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Helminth and Heavy Metals Transmission from Anaerobically Digested Sewage Sludge

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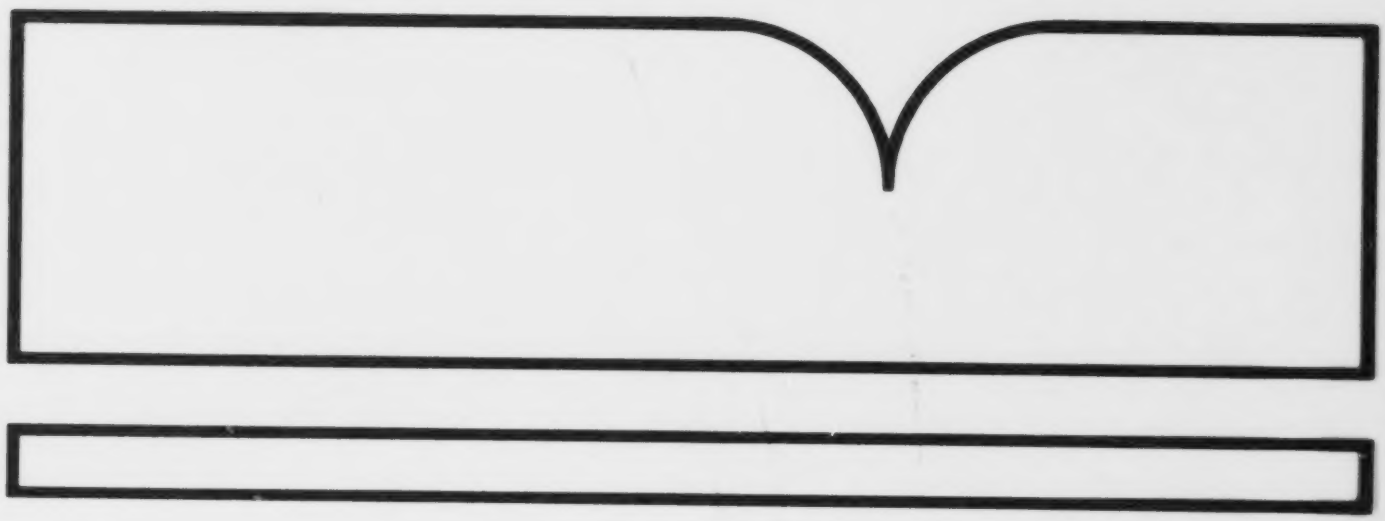

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HELMINTH AND HEAVY METALS TRANSMISSION FROM ANAEROBICALLY DIGESTED SEWAGE SLUDGE

by

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This study was conducted in cooperation with the Metropolitan Sanitary District of Greater Chicago, Chicago, Illinois, and the University of Illinois, Urbana, Illinois 61801.

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16. ABSTRACT

This report discusses a study designed to determine the practical survival and transmission of the ova of the nematode worm Ascaris sp. through a modern sewage and sludge treatment process. Four large experiments and three smaller ones involving 178 specific pathogen free (SPF) pigs were used. Transmission of Ascaris sp. from liquid anaerobically digested sewage sludge, that had been stored for several years, occurred in a few pigs in each of the four large experiments. Also, Ascaris sp. transmission from Nu-earth, a dried and stored anaerobically digested sewage sludge, also occurred in pigs. In general, ova in anaerobically digested sludge or in Nu-earth remained unembryonated until after they were exposed to the air. Within six weeks after air exposure the ova began to embryonate and became infective for pigs.

Swine were also exposed to different amounts of anaerobically digested and Nu-earth sludges containing heavy metals and organic compounds. Chemical analyses of kidneys, livers, hearts, diaphragm muscles and bones were conducted to determine uptake of heavy metals, cadmium, zinc, copper, iron, lead, chromium and nickel. Only cadmium showed significant tissue uptake. Neither control nor experimental pigs showed significant visceral fat uptake of organic compounds.

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimonies to the deterioration of our natural environment. The complexity of that environment and the interplay of its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems to prevent, treat, and manage wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, to preserve and treat public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research and provides a most vital communications link between the researcher and the user community.

In a modern society, adequate and appropriate disposal of waste materials is an important process. Human, animal, and industrial wastes accumulate in large quantities as the population grows and there is increasing demand for material goods. Part of the waste disposal problem is created by the municipal sewage system. Since human and animal wastes contribute large quantities of organic and inorganic materials to the system, a concern as to the potential transmission of organisms or materials that cause disease has become a topic of major importance. The information contained within this report has been developed through research relating to the potential transmission of disease from sewage products to animals.

Francis T. Mayo, Director
Municipal Environmental
Research Laboratory

ABSTRACT

This report discusses the findings of a study designed to determine the practical survival and transmission of the ova of the nematode worm Ascaris sp. through a modern sewage treatment process. Four large experiments and three smaller ones involving 178 specific pathogen free (SPF) pigs were used. Transmission of Ascaris sp. from liquid anaerobically digested sewage sludge, that had been stored for several years, occurred in a few pigs in each of the four large experiments. Also, transmission from Nu-earth, a dried, stored sewage sludge, also occurred in pigs which were exposed to this material by contact in the pens. In general, ova in anaerobically digested sludge or in Nu-earth remained unembryonated until after they were exposed to the air. Within six weeks after exposure to air, the ova began to embryonate and thereafter a small percentage of the ova, which embryonated became infective for pigs.

The occurrence of heavy metals in the tissues of swine exposed to the sewage by-products, anerobically digested sludge, or Nu-earth originating from a large municipality, were also studied. Chemical analyses of kidneys, livers, hearts, diaphragm muscles, and bones were conducted to determine the quantities of the heavy metals cadmium, zinc, copper, iron, lead, chromium, and nickel in the tissues following exposure of the pigs to different amounts of the sewage products. Only cadmium showed increases relating to the pig's association with increased quantities of sludge containing heavy metals. No physiological or pathological conditions, associated with exposure to the sludge material, were detected. Examination of visceral fat from control and experimental pigs indicated that there was no unusual accumulation of organic compounds including polychlorinated biphenyls (PCB's) and the insecticides heptachlor and dieldrin.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

MSDGC	-- Metropolitan Sanitary District of Greater Chicago
ADS	-- anaerobically digested sludge
NE	-- Nu-earth (dried sludge)--stored sludge from MSDGC system
T/Ha	-- metric tons per hectare
PPM	-- parts per million
PPB	-- parts per billion
PCB	-- polychlorinated biphenyls
DMT	-- dry metric tons
NBS	-- National Bureau of Standards
WSW	-- West Southwest sewage treatment plant
Sludge	-- residue left after raw sewage has been subjected to aerobic or anaerobic digestion by bacteria

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SECTION 1

INTRODUCTION

The many important aspects of the use of energy are currently issues of concern to the American public. One of the problems associated with the use of energy is that dealing with the end product that results from the wastes of society--sludge. For example, large quantities of sewage and manufacturing sludges are produced every day in the United States. The Metropolitan Sanitary District of Greater Chicago processes approximately 1.34 billion gallons (5.07 billion liters) of water (sewage) daily, from which 482 dry metric tons of solids are extracted. This material comes from 5.5 million humans and from industrial output equivalent to another 4.5 million humans. If these figures are used to extrapolate the waste disposal problem for 225 million humans in the United States, we can calculate that we must dispose of about 10,845 metric tons of dry sludge each day or nearly 4 million dry metric tons per year. Each day's sludge would fill 240 average-sized railroad coal cars. This includes the waste materials that enter small municipalities and private cesspools or septic tanks, but it does not consider the large quantities of industrial waste that must also be disposed of.

USE OF SLUDGE AS A FERTILIZER

We use great quantities of fossil fuels to produce chemical fertilizers to nourish many kinds of food-producing plants. We have not yet been forced to use fertilizers containing less concentrated nutrients but which may otherwise be acceptable fertilizers. Aerobically and anaerobically digested sludges are fertilizers that may be classed as acceptable substitutes under some conditions. In addition to their activity as fertilizers, these materials have characteristics that enable them to improve the physical nature of some soils (1,2,3). Sludges are not without fault, however, and they cannot be expected to improve growing conditions under all circumstances (4). Under appropriate conditions, sludges are highly useful products that may be used effectively in the production of food by introducing them, as supporting links, into the food chain. Use of liquid sludge, for example, as an irrigant fertilizer for forage and cereal crops is one important usage (5). Anaerobically digested sludge, containing 2% to 5% solids, is beneficial and useful in the reclamation of strip-mine spoils. Digested sludge is applied to the land by sprinklers, injection, or flood-irrigation; it is then incorporated into the soil to improve the raw soils by adding organic material to them. Later, a suitable forage grass or cereal may be planted and animals allowed to graze on the forage. Cereals may be grown and harvested by conventional methods, or livestock

may be allowed to forage, feeding on grass, stalks, ear corn, or kernels that may have been left in the field after the harvest.

HEALTH ASPECTS

The possible influence of sludges upon the health of humans and other animals is a matter of concern (6,7). With an increasing usage of sludges as fertilizers and soil conditioners in agriculture, the question as to whether disease can be transmitted to animals or to man must be addressed. The question of survival and transmission of viruses, rickettsias, bacteria, fungi, and various other parasites through the sewage treatment process has been only partially answered (8,9,10).

Numerous reports appear in the literature, some of which note the survival of parasitic organisms, but they generally fail to assess the possibilities of transmission of organisms that subsequently could produce clinical disease in man or animals (11). When one considers survival of organisms, one must carefully define the nature of the sewage. Many organisms survive quite well in raw sewage, a few survive in aerobically digested sludge, but only a very few survive in anaerobically digested sludge. Composting raw sewage or sludges raises the temperature of the compost by bacterial and/or chemical action (7,12). If the compost temperature is maintained at approximately 60 C for 30 minutes, most pathogens will be inactivated (8). Unfortunately, composting is usually not economically possible in either very small or very large sewage disposal systems. Anaerobic digestion is usually carried out at 35 to 38 C, while aerobic digestion occurs at temperatures that are near to that of the surrounding environment (18 to 23 C). A very few thermophilic bacteria (nonpathogenic) may survive short exposure to high temperatures, and a few methane producers grow best at about 54 C (8,10,12).

Most viruses, rickettsias, and other bacteria require highly specific living conditions and are incapable of surviving in the harsh environment supplied by composting or anaerobic digestion (13). Most pathogenic bacteria succumb to temperatures in the range of 50 to 70 C (8). The pasteurization process is based upon the principle of sustained heat (62 C for 30 minutes) to destroy harmful bacteria. However, some bacteria and fungi are able to produce resistant spores having shells or walls which help protect them from extremes in the environment (8). Viruses, in general, are more sensitive and usually require an intracellular environment wherein they are protected from extremes.

Some of the most resistant pathogenic organisms present in sewage are macroparasites. The encysted stages of several protozoa (Entamoeba histolytica, Giardia lamblia, etc.) may be able to withstand the chemical activities of anaerobically digesting sludge, but the high temperature (35 to 38 C) in the digesters, or 60+ C in compost will destroy the protozoans and thus eliminate possible transmission of the encysted stages (14). The lower temperatures of anaerobic digestion (35 to 38 C) do not inactivate all Ascaris ova, but the 60+ C temperature of the compost pile is lethal to them.

Researchers have made both positive and negative assumptions regarding the significance of parasite ova in sewage (11,15). Some have assumed that the very presence of ova makes them a threat to public health, while others have claimed that they are unimportant because they have not been observed to be embryonated and therefore are not infective.

Unfortunately, neither philosophy has been adequately examined by experimentation but only by hearsay. Some researchers and some popular media have condemned the use of sludge as fertilizer because of the possible dispensing of pathogens or harmful chemicals to the environment. The ova of cestodes, trematodes, nematodes, and arthropods are regularly recovered from sludge, and some are resistant to digestion. Ova of Ascaris lumbricoides of man, and a closely related form from swine, are commonly observed pathogens. Experimentally, we have determined that ova of this worm are resistant to anaerobic digestion at 38 C. Seventy-seven percent of the ova held in sludge at 38 C for 25 days and then exposed for an additional 58 days to 1% formalin and air at 22 C embryonated and became infective (16). We have recovered ova from anaerobically digested sludge from several species of parasites occurring in both man and animals. Most of the ova appear to be dead, but when placed under appropriate conditions of temperature, humidity, and oxygen, some are capable of embryonating and developing to the infective stages.

There are many reports in the literature which refer to the presence of the ova of Ascaris lumbricoides in sludge, but they fail to note how frequently the ova cause infections in animals, if at all. It has been reported that the ova of this worm are capable of remaining in the soil and becoming infective for 5 to 6 years or more (17). Other ascarid ova, such as Toxocara canis and Toxascaris leonina from dogs and cats, are as resistant as Ascaris lumbricoides (18).

The parasitic worms that have been grouped under the family ascaridae are among the most ubiquitous of parasites. In areas of the world where sanitary facilities are poor or are nonexistent, the incidence in the human population is high. Animals, such as dogs and pigs, are frequently infested. The worms that occur in man (Ascaris lumbricoides) and in swine (Ascaris lumbricoides suum) look alike, but there probably are some minute structural and physiological differences. The worms occurring in dogs and other animals are different.

There are basic characteristics of the life cycles that appear to be the same. For example, the basic life cycles of Ascaris lumbricoides of man and Ascaris lumbricoides suum from swine are the same. The life cycle of Toxocara sp. from dogs is basically the same as for Ascaris sp., but it differs in an important way in that the larvae migrate through the body differently.

The basic life cycle of Ascaris lumbricoides and A. l. suum begins when an ovum (destined to be a female worm) is produced and is passed out of the fertilized female worm that resides in the small intestine of the host. The ovum passes out in fecal material in an unembryonated state and is noninfective. If the ovum is exposed to air under the right conditions

of temperature and humidity, without being exposed to sunlight and drying, it undergoes a development in which a small worm develops within the shell. When embryonation is completed, a second stage larva is present and the ovum is infective and is said to be embryonated. A human or pig, ingesting the ovum with its food, becomes infested. When the ovum reaches the stomach and intestines, the combined chemical and physical activity initiated there cause the larvae to emerge into the intestine. The young larva burrows into the intestinal tissue and is picked up by the blood stream and is transported to the liver. After about five days in the liver, the larva migrates, via the blood, to the lungs where it remains for about another week. It then breaks out of the air passages and migrates up the tracheal tree to the pharynx and is swallowed. It passes through the stomach and comes to lie in the intestine where it develops to the adult stage, fertilization by a male worm occurs, and the cycle begins again. Fourteen to 21 days are usually required to complete this part of the cycle. It requires about three weeks, under ideal conditions, for the ovum to embryonate. The period after ingestion of the infective ovum until ova are produced by the new worm is about 49 to 62 days. Assuming ideal conditions, the complete life cycle from unembryonated ovum to unembryonated ovum, would be 70 to 83 days.

In studies conducted at the "Prairie Plan" sludge disposal site in Fulton County, Illinois, a part of the Metropolitan Sanitary District of Greater Chicago disposal system, we have observed ova from parasitic worms in anaerobically digested sludge that had been stored for several years. Calculations have shown that, on the average, the overhead or the incorporation irrigation methods used to dispense anaerobically digested sludge deposits approximately 3-1/2 million ova of Ascaris spp. per hectare on/in the land during the irrigation season. This application of ova is derived from an approximate 18 metric ton/hectare application of sludge. A small number of these ova are known to be either already embryonated or are capable of embryonating once they have been exposed to the air. These parasites are significant pathogens in themselves because they are capable of migrating through the body tissues of many animals, including man (19). Furthermore, during their migrations in the tissues, they open pathways through which secondary invasion of the tissues by bacteria, viruses, or fungi may occur (13).

Because of these resistant helminth ova in conventional stabilized sludges, the potential hazard to the health of humans and animals present in the environment needs to be investigated. Contamination of foodstuffs for animals and man by application of sludges to agricultural lands is a potential threat to the health of the subjects. Since the ova of Ascaris lumbricoides are both resistant and common, the subject of this study was to observe their survival through the sewage treatment cycle and to determine the potential for natural transmission to a susceptible host.

Because of the nature of the proposed study, which involved exposure of experimental animals to anaerobically digested sludge that also contains heavy metals, it was also proposed to determine the levels of some heavy metals and organic compounds in tissues of pigs exposed to the sludges. Valuable information relating to the possible uptake of these

elements or compounds from the environment with subsequent incorporation into the plants, animals, and the food chain was obtained.

One objective of this study was to determine whether there was survival and natural transmission of the resistant parasite (Ascaris sp.) ova, which were dispersed on agricultural lands in anaerobically digested sludge, to swine, a natural host. A second objective was to determine the rate of embryonation and survival of the ova in anaerobically digested sludge and/or other residues (e.g., Nu-Earth). A third objective was to determine the levels of heavy metals in the soil, feed, and tissues of experimental animals (swine) exposed to anaerobically digested sludge applied to agricultural lands at different levels.

SECTION 2

CONCLUSIONS

Exposure of swine to different levels of anaerobically digested sludge from a large municipality has shown that a potential for transmission of the nematode Ascaris sp. exists. The results of this study show that some ova from this parasitic worm are able to withstand the rigorous treatment of a modern sewage treatment plant. The exposure of swine to anaerobically digested sludge was infinitely greater than a normal human exposure would be, yet, the levels of infestation with Ascaris sp. were low. With ova present at an approximate level of 4000 per square meter of surface, embryonation of some ova occurred, some were ingested by pigs and eventually caused light infestations in the gastrointestinal tracts. Although these worms did not cause detectable pathology in the animals they infested, a potentially serious parasite problem could develop in a swine herd continuously exposed to anaerobically digested sludge. This could occur not because of the immediate damage caused by worms transmitted from the sludge, but because of the potential for build-up of numbers of ova in the environment as sexually mature female worms, originating from ova in the sludge, began to lay eggs which would "seed" the environment. Subsequent swine, exposed to the area, would be exposed to much greater numbers of ova, and a potential parasite problem could develop into a serious actual problem.

Under the conditions of this study, the health of the animals did not appear to be threatened by the parasites transmitted through the sludge. It is not likely that man would become seriously parasitized from exposure to anaerobically digested sludge for at least two reasons. First, the degree of exposure would be extremely low (at least under current sanitary conditions in the United States), and secondly, because the ova commonly seen in sludges from municipalities in the U.S. may be swine forms not readily transmissible to man.

In addition to the potential parasite problem that could develop in swine, the question of the disposition of heavy metals in sewage sludges is addressed. The results of this study have shown that cadmium was the only one of seven heavy metals which accumulated to a significant degree in tissues of swine exposed to sludge containing heavy metals. Of the 5 tissues examined for heavy metals, only kidney and liver accumulated cadmium at levels greater than in controls. Although the accumulation in these organs was statistically greater than in controls, the accumulation was far below a lethal level. An estimated 200 ppm of cadmium (wet weight) reportedly must be accumulated in the human kidney before evidence of toxicity begins to appear. It is generally assumed that kidneys of

other animals would show toxicity beginning at about the same level. The highest level observed in swine kidney tissue in this study was 11.5 ppm on a dry weight basis or 3 ppm wet weight. This quantity of cadmium had no immediate effect on the animal's health. If one uses the data on accumulation of cadmium in the kidneys of swine observed in this study, one can calculate that it would require more than 22 years of continuous exposure to dried sludge (living in pens filled with Nu-earth containing 191 ppm Cd) before 200 ppm Cd would be accumulated in the kidney of the pig. The normal life span of a "feeder" pig is 6 to 7 months.

The presence of polychlorinated biphenyls and the organic insecticides heptachlor and dieldrin in tissues of experimental animals, at levels comparable to controls, indicates that these organic compounds did not appear to be an immediate threat to animal health under the conditions of these studies. The facts revealed herein suggest that serious exposure to anaerobically digested sludge, containing organic compounds at levels described in this study, would cause no significant detrimental effect on the animals.

SECTION 3

RECOMMENDATIONS

An area not clarified in this study deals with the survival of the human ascarid (Ascaris lumbricoides) and its potential transmission to swine. In the minds of parasitologists, there is disagreement as to whether the human ascarid, Ascaris lumbricoides, and the swine ascarid, Ascaris lumbricoides suum, are the same. Some believe they are cross transmissible, while others disagree. One cannot distinguish one species from the other on the basis of the appearance of the ova. Ova extracted from sewage sludges are even more difficult to differentiate. An experiment was reported herein which was designed to determine whether human ascarids were transmitted to swine in a natural environment. In the experiment described, transmission by natural means failed to occur. Since ova of ascarids are among the organisms most resistant to the sewage digestion process, it becomes increasingly important to determine whether, in fact, there is a threat to the human population from this parasite. It was shown that ova in liquid anaerobically digested sludge and in dried sludge (Nu-earth) are potential sources of parasite infestation in swine, but we do not know whether humans can become infested. Because we cannot use human subjects for experimentation, we must study further the natural relationships between human and swine ascarids in sewage sludges. In order to clarify this relationship and the entire theory that Ascaris sp. ova in sludge is a threat, a specific study dealing with the potential transmission of human Ascaris to swine, etc., should be conducted.

The application of liquid or partially dehydrated sludges, that contain ova of Ascaris sp., presents a potential source of infestation to swine. The results of this study show that natural infestations with Ascaris sp. can occur in swine that have been in close association with these sludges. Although initially infestations are of low level if sludges are repeatedly applied to soils, the potential for greater levels of infestation increase. Animals repeatedly or continually exposed to a sludge-treated environment could develop severe parasitism. If such conditions are anticipated on a farm, it would be wise for the farmer to include an anthelmintic compound in the feeding or management program.

Under the conditions described in this study wherein pigs were naturally exposed to anaerobically digested sludges, uptake and accumulation of heavy metals did not appear to be particularly exceptional. The 9.8 ppm dry weight accumulation of cadmium in the kidneys of pigs living in the Nu-earth for four months, for example, represents a worst case situation. On a wet weight basis, this would reduce to less than 3 ppm, far below the 200 ppm generally referred to as the level at which

pathology may begin to occur. Although there was a statistical difference between control and experimental animals in terms of accumulation of some heavy metals, the levels were low and caused no detectable pathological changes. These results indicate that sludges, of the type used in these experiments, could be utilized for fertilizer or soil improvement without restrictive regulations concerned with potential transmission of animal diseases.

SECTION 4

MATERIALS AND METHODS

METHOD FOR SECURING SPECIFIC PATHOGEN-FREE (SPF) PIGS

All pigs used in this study were produced and raised under specific pathogen-free conditions at the Dixon Springs Agricultural Center Laboratory of the University of Illinois. Initially, young gilts of approximately six months of age were selected from the "closed" herd at the Center. Beginning approximately two months after breeding, each of the gilts was given three therapeutic doses of dichlorovos, over a three-week period, to insure that any intestinal helminths that might have been present were removed. The gilts were then placed in isolation rooms and were given a complete feed, ad libitum, which contained 0.0013% by weight of the anthelmintic Hygromycin (Elanco Products). The Hygromycin was maintained in the ration throughout the remainder of the gestation period. After parturition, the sows were maintained on the Hygromycin-treated feed, and the baby pigs were allowed to nurse ad libitum and to ingest medicated feed. The sows were removed from the pens when the young pigs were approximately 6 weeks old. The young SPF pigs were fed a high-protein diet (16%) containing Hygromycin for 7 to 10 days before they were shipped via truck to the Fulton County, Illinois, experimental site.

During the period of confinement of the gilts and young pigs, periodic fecal examinations were made to ascertain whether any parasites were present in the intestines of any of the animals.

After weaning, the young pigs, weighing 35 to 40 pounds, were loaded into steam-cleaned trucks in which fresh, clean bedding was added and were transported approximately 300 miles to the experimental site in Fulton County, Illinois. The site selected for the study was unique inasmuch as it had been part of a strip-mine spoil that had been graded for agricultural planting. It had never had animals grown on it since it had been mined. No domesticated animals had been on it for the past 50 years. After arrival at the experimental site, no medicated feed was given. Forty young SPF pigs, used in each of four experiments, were transported to the experimental site in the same manner.

In experiment 1, the 40 young pigs were randomly dispersed into eight pens each containing five pigs. The second, third, and fourth experiments were conducted with the same number of pigs in the same general location. Each pen, in which five pigs were initially placed, was approximately 9.8 x 14.6 m and was made of steel mesh wire panels held in place with steel posts driven into the ground (see Figures 1 and 2). There was an

approximate 3 m buffer zone between pens. Each pen contained a new self-watering tank device, a self-feeder, and a shelter, all made of galvanized steel.

DISPENSING SLUDGE TO PENS

Experiment 1 (1978)

Anaerobically digested, stored sludge was applied from a tank truck equipped with pump and hoses (Figure 1) and was spread as liquid sludge to the surface of the experimental pens. The first application of sludge was made to the soil in May, one year before the pigs were introduced into the pens, and the second seven months before introduction of the pigs to the pens. This was believed to be sufficient time for ova to embyronate in the sludge. Furthermore, it would be a test to determine the effects of overwintering on the ova. Pen 1 served as a control and no sludge was added at any time during the experiment. Pen 2 was treated with liquid anaerobically digested sludge equivalent to 22 dry metric tons per hectare. Pen 3 was treated with the equivalent of 33 dry metric tons of liquid anaerobically digested sludge per hectare. Pen 4 received the equivalent of 65 dry metric tons of liquid anaerobically digested sludge per hectare. Pen 5 received the equivalent of 22 dry metric tons of liquid anaerobically digested sludge per hectare plus 567,000 unembryonated ova of Ascaris lumbricoides suum added to the liquid sludge prior to its application to the soil in the pen. Pen 6 had the equivalent

Figure 1. Application of liquid sludge to pens in which young pigs were later kept. Solid content of sludge was from 4.0% to 5.5%.

of 22 dry metric tons of anaerobically digested sludge plus 567,000 of A. l. suum embryonated ova placed in the sludge prior to application to the soil. Pen 7 received no anaerobically digested sludge but did receive 567,000 unembryonated ova of A. l. suum applied in water dispersed by hand sprayer onto the soil. Pen 8 received 567,000 embryonated ova of A. l. suum dispersed in the same manner, no sludge.

The ova added to the sludge were recovered from gravid female worms and were embryonated in 1% formalin solution aerated with an aquarium pump. Unembryonated ova were retained in water in the refrigerator until dispensed. Both embryonated and unembryonated ova were taken from a common mixture of ova.

Fecal samples were collected several times from the sows and pigs before the experiment started. Random fecal samples were taken from the pens beginning approximately 3 weeks after the experiment started. One pig, selected at random from each pen, was necropsied 3 weeks after being placed in the pens, and liver and lung tissue were baermannized to recover any migrating larvae.

Experiment 2 (1978)

This experiment consisted of eight pens each containing five pigs. Pens 9, 10, and 11 received no sludge at any time and served as controls for the rest of the experiment. Pen 12 received 18 dry metric tons of Nu-earth equivalent to 1,134 dry metric tons of anaerobically digested sludge per hectare (see Figure 2) immediately prior to the introduction of the pigs. The Nu-earth contained 25% to 35% water and had been stock-piled near Chicago, Illinois, for an unknown time but probably 6-10 years. Each of four pens, numbers 13, 14, 15, and 16, received liquid anaerobically digested sludge taken from the Fulton County storage lagoon (at least 1 year old but probably 3 to 10 years old) equivalent to 25 dry metric tons per hectare. Sludge was spread in the pens as indicated for experiment 1. No ova, either embryonated or unembryonated, were seeded into the sludge. Pens 13 through 16 were located approximately 154 m away from pens 9 through 12. Pigs were placed in pens immediately ater the liquid sludge was placed in the pens.

Experiment 3 (1979)

This experiment used a total of 40 pigs placed in the same pens that were previously used for experiment 2. This was a replication of experiment 2 and occurred during the summer of 1979. No additional sludge or Nu-earth was added to the pens.

Experiment 4 (1979)

This experiment was initiated in a new area approximately 465 m away from the site where the previous three experiments had been conducted. Eight pens, of the type previously described, were constructed with a 3 m buffer zone between each pen. Approximately 18 metric tons of partially dehydrated sludge (Nu-earth), taken directly from the drying beds of the

Figure 2. Application of "Nu-earth" to pen in which pigs were later kept. Nu-earth was semi-dry --about the consistency of a moist garden soil.

West-Southwest plant of the Metropolitan Sanitary District of Greater Chicago, was transported 180 miles by truck to the experimental site and was spread in pen 23. A similar quantity of dehydrated dried sludge (Nu-earth) was transported from a storage pile (several years in storage) of the Metropolitan Sanitary District of Greater Chicago and was placed in pen 21. One-thousand gallons (3785 liters) of anaerobically digested sludge, containing approximately 3-1/2% solids (about the equivalency of 10 DMT/Ha), was taken directly from the barge, without prior storage in a lagoon, and was spread on the soil in each of pens 17 and 19. A control pen was placed between each experimentally treated pen; that is, between pens 17 and 19, 19 and 21, 21 and 23, and on the remaining side of pen 23 (pen 24). Pigs were placed in the pens one week after the application of the Nu-earth or liquid sludge.

In each of the experiments described, the young pigs were allowed to live in a natural habitat under natural conditions receiving water and feed in a normal environment similar to that which would exist on a pig farm.

ADDITIONAL STUDIES

Supplemental Study 1

An additional study involved seven or eight pigs in two pens of the same size indicated above. In this study SPF pigs, of the type indicated previously, were placed in pens in which unembryonated or embryonated Ascaris lumbricoides ova, originating from human patients in Medellin, Colombia, were dispensed into the pen by hand sprayer (see Figure 3). Each pen received approximately 4,000 embryonated or unembryonated ova of A. lumbricoides per square meter, for a total of 572,000 ova per pen. Seven or eight SPF pigs were placed in the pens one week before the ova were seeded into the pens. Each pen initially contained some grass/legume forage (see Figure 3). Pigs then had intimate contact with the soil, vegetation, etc., in the pens for approximately four months. They were then necropsied and examined as described under necropsy procedures.

Figure 3. Dispensing ova of Ascaris lumbricoides to vegetation and soil of pens in supplemental study 1.

Supplemental Study 2

In this study, three 13 to 15 kg SPF pigs were fed liquid anaerobically digested sludge as a part of the regular diet to determine whether ova present in the sludge would produce infections in the pigs. Ten gallons (37.8 l) of fresh liquid anaerobically digested sludge (2.12% solids) were collected at the WSW plant of the MSDGC and was brought to the laboratory. Aeration of the sludge was started, using aquarium pumps, and continued for 104 days at room temperature (24 C). After 104 days of aeration, 400 ml of the sludge was fed daily, in the ration to each of the pigs for 30 days. Seventy-seven days after they first received sludge in the diet, they were necropsied and the liver, lungs, and intestines were examined for Ascarid worms. The remainder of the carcasses were carefully examined for other parasites. Pieces of liver, kidney, diaphragm, and heart were fixed in 10% formalin for possible histopathological examination. Additional samples of these organs were taken for possible future heavy metals analysis.

Supplemental Study 3

This study was initiated during the summer of 1979 to determine the rate of embryonation of swine ascarids in soil treated with various quantities of liquid anaerobically digested sludge. A 4- x 10-m plot of soil that was known to have been free of any exposure to animals for the past 15 years was identified. The plot was cultivated to a depth of 25 cm with a rototiller and was then divided into eight 0.5- x 0.5-m plots. Each plot was delineated by a metal edging around the periphery to seal off the plot (see Figure 4). Fresh, anaerobically digested sludge (4.7% solids) was applied to some plots as indicated below. Two plots received Nu-earth that had been dried at 40 C for 48 hr then ground to a uniform consistency before use in the plots.

Adult female Ascaris l. suum were used as the source of ova. The worms were dissected and the proximal portion of the vagina and 2 cm of the uterine branches were recovered. The ova were extracted and then agitated in 0.5 N NaOH for a few minutes to remove the sticky coating, then washed in water and dispensed on the plots as follows:

Plot 1 = untreated control; no ova, no sludge.
Plot 2 = 2.0×10^6 unembryonated Ascaris ova applied to the soil surface in a water spray.
Plot 3 = 2.0×10^6 unembryonated Ascaris ova seeded into 3 liters of sludge (4.7% solids) applied to the soil surface.
Plot 4 = 2.0×10^6 unembryonated Ascaris ova seeded into 6 liters of sludge (4.7% solids) applied to the soil surface.
Plot 5 = 2.0×10^6 unembryonated Ascaris ova seeded into 3 liters of sludge (4.7% solids) incorporated into the soil to a depth of 10 cm.
Plot 6 = 2.0×10^6 unembryonated Ascaris ova seeded into 6 liters of sludge (4.7% solids) incorporated in the soil to a depth of 10 cm.

Plot 7 = 2.0 x 10^6 unembryonated Ascaris ova seeded into 1 kg of Nu-earth applied to the soil surface.

Plot 8 = 2.0 x 10^6 unembryonated Ascaris ova seeded into 2 kg of Nu-earth applied to the soil surface.

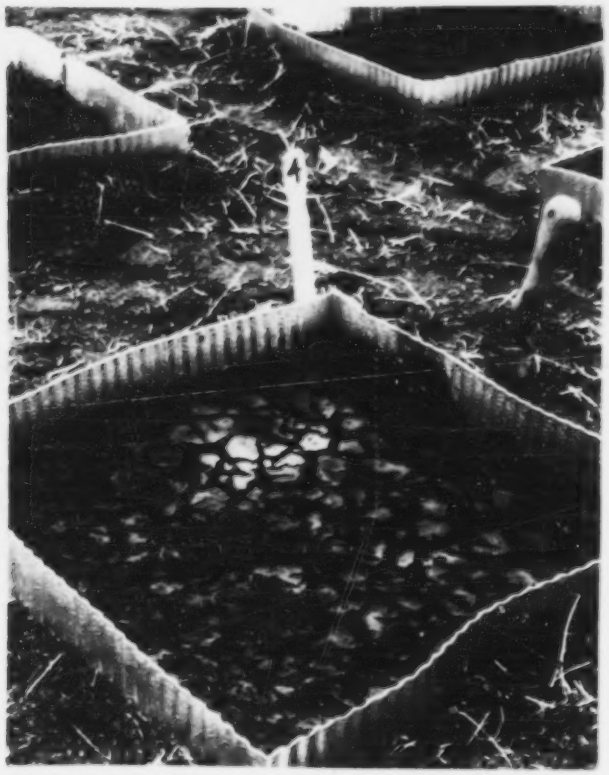

Figure 4. Typical plot showing area treated with sludge to determine the embryonation of Ascaris ova in supplemental experiment 3. Summer 1979.

Soil samples of 100 to 150 g from each plot were examined weekly for 7 weeks for Ascaris ova, and the extent of embryonation was estimated. Recovery of ova from soil samples was by a modified Arther method (20). The soil sample was passed through 12-, 20-, and 40-mesh screens, then 3 to 4 g were liquified in 0.1-N NaOH and then poured through a 60-mesh screen. Additional 0.1-N NaOH was added, and the mixture was centrifuged in 50-ml tubes. After the sediment was washed clean in 0.1-N NaOH solution, a portion of the sediment was resuspended in Sheather's sugar solution to float the ova to a coverslip. The coverslip was then removed and was placed on a microscope slide and examined to determine the stage of embryonation.

NECROPSY PROCEDURE

After approximately four to four and one-half months in each of the major experiments, each of the pigs was killed by electrical stunning followed by bleeding from the jugular vein. Following exsanguination, each pig was opened ventrally and the internal organs were removed. The lungs, liver, kidneys, mesenteries, etc., were each examined grossly for parasitic organisms or for lesions or other evidence of abnormality. The gastrointestinal tract was opened along its entirety and was meticulously examined for gross stages of parasitic worms. Because of the quantities of material involved, it was not possible to examine the G.I. tracts microscopically.

The muscles, joints, and other areas of each animl, such as the body cavity, were examined for evidence of tissue parasites or other lesions. The brains were not examined. The residue, after necropsy, was incinerated.

DETERMINATIONS OF HEAVY METALS IN TISSUES OF SWINE EXPOSED TO ANAEROBICALLY DIGESTED SLUDGE

In each of the studies indicated above, tissues from necropsied animals were recovered for possible future examination for heavy metals. Since cost was a determining factor, only animals from experiment 1 and part of experiment 2 were selected for determination of heavy metals. At necropsy pieces of muscle (diaphragm), heart, liver, kidney, and neck bone were recovered for chemical analysis. Each tissue was removed and wrapped in aluminum foil, labelled, and transported to the laboratory for storage at -10 C until ready for analysis. Tissues were removed from each of the 32 pigs in experiment 1 and from 12 additional pigs not exposed or exposed to liquid sludge or Nu-earth in experiment 2.

All tissues were kept frozen until needed for processing for chemical analysis. Frozen tissues were removed from the freezer, and after partial thawing, were sliced into 6 mm thick sections with stainless steel knives. The frozen tissues were refrozen and then placed in a lyophilizer for 48 hr. After 48 hr, 2 g of the dried tissue was used for digestion. The digestion procedure was as follows: 2 g of tissue was placed in a 24 mm diameter corex glass tube and was initially treated with 5 ml of double distilled nitric acid. Digestion was allowed to proceed overnight or approximately 16 hr at room temperature (24 C). An additional 5 ml of acid was added to the tube, and the tube was placed in a heating block, gradually heated over a period of 8 hr to 95 C, and then allowed to digest at 95 C overnight. Following digestion and evaporation in a hood, the residual tissue-acid mixture was further treated with an additional 10 ml of nitric acid and again heated from about 40 C to 95 C during an 8-hr period and again left to continue digesting overnight. At the end of the second digestion period, 10 ml of purified hydrogen peroxide was added to the residual material in the tube, heated to 80 C, and again allowed to digest for approximately 16 hr. At the end of this digestion period, the residue in the tube was dissolved in 2.5 ml of 1 $\underline{N}$ HNO_3 and was then

diluted with double distilled (over glass) water to a volume of 25 ml. A portion of this solution was then analyzed in a Perkin-Elmer model 403 spectrophotometer by conventional atomic absorption solution aspiration techniques. When concentrations were less than 1 ppm, a Perkin-Elmer model 360, fitted with an HGA model 2100 graphite tube furnace equipped with an AS-1 automatic sample changer, was used. All measurements were made in duplicate with appropriate background correction applied. All readings were compared to predetermined standards and calculations were then made to determine the quantities of heavy metals on a dry weight basis.

This procedure was used for all digestions, including bone. The results were compared to the National Bureau of Standards determinations for bovine liver. All were within the range indicated by the NB Standard Reference Material #1577 bovine liver, except for copper in the swine liver tissue.

SECTION 5

RESULTS

EXPERIMENT 1

The results of experiment 1 are shown in Tables 1-3. Table 1 shows the total quantities of sludge deposited in each of the 8 pens used in experiment 1 with the dates of application. Table 2 shows the number of ova of Ascaris sp. naturally present in the sludge dispensed in the pens of experiment 1 and also the number of embryonated or unembryonated ova "seeded" into the pens. Table 3 shows the number of ova observed in fecal samples taken from animals after they were placed in the pens. No ova were recovered from any fecal samples until one week before necropsy. It also shows the number of larvae recovered from liver or lungs of a young pig necropsied from each of the eight pens in experiment 1.

TABLE 1. QUANTITIES OF SLUDGE APPLIED TO INDIVIDUAL PENS IN EXPERIMENT 1

Pen No.	April 1977 (4.7% solids)		October 1977 (4.7% solids)		Total applied	
	liters	Dry metric tons/Ha	liters	Dry metric tons/Ha	liters	Dry metric tons/Ha
1	0		0		0	0
2	3785	(11)	3785	(11)	7570	22
3	7570	(22)	3785	(11)	11355	33
4	15140	(43)	7570	(22)	22710	65
5	7570	(22)	0	0	7570	22
6	7570	(22)	0	0	7570	22
7	0	0	0	0	0	0
8	0	0	0	0	0	0

TABLE 2. NUMBER OF OVA OF ASCARIS SP. NATURALLY PRESENT IN SLUDGE AND NUMBER "SEEDED" IN SLUDGE. EXPERIMENT 1

	May 1977		October 1977	
Pen No.*	No. of ova naturally present in unseeded sludge	No. seeded ova	No. of ova naturally present in unseeded sludge	No. seeded ova
1	0	0	0	0
2	190,000	0	190,000	0
3	380,000	0	190,000	0
4	757,000	0	567,000	0
5	380,000	567,000 unembryonated	0	0
6	380,000	567,000 embryonated	0	0
7	0	567,000 unembryonated	0	0
8	0	567,000 embryonated	0	0

*Each pen contained 145 square meters of soil surface.

+In unseeded sludge, each liter contained approximately 50 unembryonated ova of Ascaris sp.

TABLE 3. ANALYSIS OF YOUNG PIGS, AFTER 3 WEEKS EXPOSURE TO SLUDGE, FOR OVA IN FECAL SAMPLES, LARVAE RECOVERED FROM LIVER OR LUNG TISSUE, OR WHITE SPOTS IN LIVER. EXPERIMENT 1

Pen & Pig No.	Fecal 5/11/78	Fecal 6/1/78	Liver spots 6/1/78	Lung larvae 6/1/78
1 (38)	-	-	-	-
2 (32)	-	-	-	-
3 (29)	-	-	-	-
4 (24)	-	-	-	-
5 (19)	-	-	-	-
6 (13)	-	-	+	1
7 (10)	-	-	-	-
8 (3)	-	-	+	1

Table 4 shows the occurrence of immature or adult Ascaris sp. in the gastrointestinal tracts of 32 pigs at necropsy and indicates the presence of gross pathology in the liver (white spots) or lungs (pneumonitis). Fourteen of the 32 pigs in experiment 1 contained ascarids in the intestines. The majority of the worms were recovered from pigs inhabiting pens 5 through 8 which had been seeded with either unembryonated or with embryonated ova. However, one pig, number 28 from pen 3, contained ascarids in the intestine. This was a single infection originating from the association of the animal with sludge, applied at the rate of 33 dry metric tons/hectare, for a period of approximately 4 months.

The pigs infected in pens 5 and 6 show that sludge was not lethal to the ova when they were "seeded" into the sludge prior to dispensing in the pens. Fourteen of 16 pigs exposed to ascarid ova seeded into sludge or no sludge became infected, while only 1 of 16 pigs became infected from exposure to unseeded sludge or natural soil. This indicates that Ascaris sp. ova, present in sludge, were capable of causing infestations in susceptible pigs.

The 14 pigs which became infected when exposed to sludge that had been seeded with ova one year earlier showed that the sludge had little harmful effect on the ova and did not prevent embryonation of unembryonated ova. It also showed that the ova could remain infective for at least one year after exposure to sludge. Although the ova used to seed the sludge had been taken from the uteri of adult female worms, as previously indicated, the need for hardening did not appear to be a limiting factor to the survival of the ova under natural conditions of exposure in the sludge.

EXPERIMENT 2

Table 5 shows the quantity of sludge applied per pen in experiment 2. Pigs in pens 9, 10, and 11 served as unexposed controls, and these pens were not treated with sludge. As shown, pen 12 was treated with 18 metric tons (equivalent of 1134 dry metric tons/ha) of dehydrated sludge, Nu-earth, and each of pens 13 to 16 received the equivalent of 25 dry metric tons per hectare applied as liquid sludge. No ova were recovered from the fecal samples taken from pigs 3 weeks after the start of the experiment (Table 6). No larvae were recovered from young pigs necropsied 3 weeks after the start of the experiment. Table 7 shows the occurrence of immature or adult worms in the gastrointestinal tracts of 32 pigs at necropsy with an indication of gross pathology in the liver (white spots). Ascarids were recovered from 2 of 4 pigs in the group that was exposed to Nu-earth (pen 12) and 3 of 4 pigs in one of the groups (pen 13) which had been treated with the equivalent of 25 dry metric tons of sludge. Three of 4 control pigs in the pen immediately adjacent to the pen treated with Nu-earth contained ascarids in the intestine. This suggests a contamination from pen 12 (Nu-earth) or the possible transmission on the shoes of caretakers or transmission by birds commuting between the two pens. A distance of approximately 3 m was thought to have been adequate space between the pens to prevent any possible transmission.

TABLE 4. OCCURRENCE OF IMMATURE OR ADULT WORMS IN THE GASTROINTESTINAL TRACTS OF 32 PIGS AND THE PRESENCE OF GROSS PATHOLOGY IN LIVER (WHITE SPOTS) OR LUNGS (PNEUMONITIS) AT NECROPSY.* EXPERIMENT 1

Pen and Pig No.		Liver spots	Lungs	Ascaris in G.I. tract
1	(36)	-	-	-
Control	(37)	-	-	-
	(39)	-	-	-
	(40)	-	-	-
2	(31)	+	-	-
380,000	(33)	-	-	-
ova	(34)	-	-	-
	(35)	-	-	-
3	(26)	-	-	-
570,000	(27)	-	-	-
ova	(28)	-	-	3
	(30)	-	-	-
4	(21)	-	-	-
1,324,000	(22)	-	-	-
ova	(23)	-	-	-
	(25)	-	-	-
5	(16)	+	-	10
947,000	(17)	+	-	5
ova	(18)	+	-	3
	(20)	-	-	5
6	(11)	-	-	1
947,000	(12)	+	-	10
ova	(14)	+	-	3
	(15)	+	-	98
7	(6)	+	-	2
567,000	(7)	+	-	4
ova	(8)	+	-	2
	(9)	+	-	-
8	(1)	+	-	13
567,000	(2)	+	-	-
ova	(4)	+	-	3
	(5)	-	-	1

*Necropsied 8/29/78 and 9/1/78, 16 weeks after introduction into pens variously treated with sludge and/or ova.

TABLE 5. QUANTITY OF SLUDGE APPLIED TO PENS. EXPERIMENT 2

Pen No.	Liters* (5.5% solids)	Equivalent dry metric tons/ha
9	0	0
10	0	0
11	0	0
12	**	1134
13	7570	25
14	7570	25
15	7570	25
16	7570	25

*Each liter of sludge contained approximately 50 ova of Ascaris, Toxocara, and Trichuris per liter. (Approximately 380,000 ova of each species in the sludge.)

**Approximately 9,006,000 Ascaris sp. ova were present in 18 dry metric tons applied. Viability is unknown.

TABLE 6. ANALYSIS OF YOUNG PIGS AFTER 3 WEEKS FOR OVA IN FECAL SAMPLES, LARVAE RECOVERED FROM BAERMANNIZED LIVER AND LUNG TISSUE OR WHITE SPOTS ON LIVER. EXPERIMENT 2

Pen & Pig No.	Fecal ova 7/17/78	Fecal ova 8/9/78	Liver spots 8/9/78*	Presence of larvae in lungs or liver 8/9/78*
9	-	-	-	-
10	-	-	-	-
11	-	-	-	-
12	-	-	-	-
13	-	-	-	-
14	-	-	-	-
15	-	-	-	-
16	-	-	-	-

*Pigs necropsied approximately three weeks after start of experiment (8/9/78).

TABLE 7. OCCURRENCE OF IMMATURE OR ADULT WORMS IN THE GASTROINTESTINAL TRACTS OF 32 PIGS AND THE PRESENCE OF GROSS PATHOLOGY IN LIVERS (WHITE SPOTS) OR LUNGS (PNEUMONITIS) AT NECROPSY.* EXPERIMENT 2

Pen & Pig No.		Liver spots	Lungs pneumonitis	Ascarids present
9	P45F	-	-	-
Control	P46F	-	-	-
	P47F	-	-	-
	P48F	-	-	-
10	P35F	-	-	-
Control	P36F	-	-	-
	P37F	-	-	-
	P38F	-	-	-
11	P25F	-	-	-
Control	P26F	-	-	1
	P27F	-	-	1
	P28F	-	-	2
12	P15F	-	-	1
Nu-earth	P16F	-	-	1
	P17F	-	-	-
	P18F	-	-	-
13	K45	-	-	1
Liquid	K46	-	-	2
Sludge	K47	-	-	1
	K48	-	-	-
14	K35	-	-	-
Liquid	K36	-	-	-
Sludge	K37	-	-	-
	K38	-	-	-
15	K25	-	-	-
Liquid	K26	-	-	-
Sludge	K27	-	-	-
	K28	-	-	-
16	K15	-	-	-
Liquid	K16	-	-	-
Sludge	K17	-	-	-
	K18	-	-	-

*Necropsied 11/14/78 and 11/17/78.

EXPERIMENT 3

Experiment 3 was a replication of experiment 2 conducted in 1978. The same pens were used without additional anaerobically digested sludge being applied, except pen 11 (control) was moved approximately 155 m away from pen 12 (containing Nu-earth). There was no additional Nu-earth placed in pen 12. Although 40 pigs were started on the study in April 1979 continuing to August 1979, six pigs were lost in the last week before necropsy because of extremely hot and humid weather. Thirty-four of the 40 pigs survived to necropsy. Thirteen were control pigs, two were exposed to Nu-earth, and 19 were exposed to soil which had been treated with the equivalent of 25 dry metric tons of liquid anerobically digested sludge one year earlier. Seven of the latter had ascarids in the intestines at necropsy. Pigs in pen 15 were negative and only one in each of pens 14 and 16 was infested. All five pigs in pen 13 were infested, probably because of a "seeding" of the environment by three infested pigs in this pen a year earlier (Table 7). These infestations would be due to the survival of ova in the environment for a year. Both of the survivors that were exposed to Nu-earth had ascarids in the intestines. One of 13 control pigs contained one immature male ascarid in the intestine (Table 8). Contamination of the environment could have occurred by dust, by contamination from birds moving back and forth between pens, or by possible contamination on the feet of caretakers. However, caretakers were cautioned never to enter into control pens in a manner that would cause contamination. Nevertheless, accidental contamination apparently occurred in one pig. No young pigs were killed early in the study to examine the liver and lungs for the presence of larvae in this experiment. Since it had already been shown that larvae were present and migrating in a previous experiment, it was believed that it would be counterproductive to remove eight experimental pigs from the study in an attempt to indicate the presence of additional larvae.

EXPERIMENT 4

This experiment was conducted in an area removed from the previous three experiments by about 500 m, across a clean alfalfa field where no domestic animals had been before (see Figure 5). Two of the 40 pigs used in this experiment were lost in the week prior to necropsy because of extreme hot and humid weather. Seventeen of 19 control pigs survived to necropsy. Twenty-one of 21 pigs exposed to Nu-earth or to anerobically digested liquid sludge survived to necropsy. Table 9 shows the results of the necropsy findings relative to the presence of ascarids in the gastrointestinal tracts and of the presence of "milk spots" in the liver or pneumonitis in the lungs. As shown, none of the five pigs exposed to freshly collected Nu-earth, taken directly from the drying beds of the Metropolitan Sanitary District of Greater Chicago, became infested with ascarids (pen 23). Two of five pigs exposed to Nu-earth from the long term storage area of the Metropolitan Sanitary District of Greater Chicago system were infested with ascarids at necropsy (pen 21). Seven of 11 pigs exposed to fresh sludge, taken directly from the anaerobic digesters of the WSW plant of the MSDGC at Stickney, Illinois, and transported by barge

TABLE 8. OCCURRENCE OF IMMATURE OR ADULT WORMS IN THE GASTROINTESTINAL TRACTS OF 34 PIGS AND THE PRESENCE OF GROSS PATHOLOGY IN LIVERS (WHITE SPOTS) OR LUNGS (PNEUMONITIS).* EXPERIMENT 3

Pen & pig no.		Liver spots	Lungs pneumonitis	Ascarids present
9	P35F	-	-	-
Control	P36F	-	-	-
	P37F	-	-	-
	P38F	-	-	-
	P39F	-	-	-
10	P25F	-	-	-
Control	P27F	-	-	-
	P28F	-	-	-
	P29F	-	-	-
11	P15F	-	-	1
Control	P17F	-	-	-
	P18F	-	-	-
	P19F	-	-	-
12	P05F	-	-	2
Nu earth	P06F	+	-	1
13	P45F	-	-	1
liquid	P46F	-	-	1
sludge	P47F	+	-	3
	P48F	-	-	1
	P49F	-	-	1
14	P35F	-	-	-
liquid	P36F	-	-	-
sludge	P37F	-	-	-
	P38F	-	-	1
15	P25F	-	-	-
liquid	P26F	-	-	-
sludge	P27F	-	-	-
	P28F	-	-	-
	P29F	-	-	-
16	P15F	+	-	2
liquid	P16F	-	-	-
sludge	P17F	-	-	-
	P18F	-	-	-
	P19F	-	-	-

*Necropsied 8/7/79 and 8/10/79.

TABLE 9. OCCURRENCE OF IMMATURE OR ADULT WORMS IN THE GASTROINTESTINAL TRACTS OF 38 PIGS AND THE PRESENCE OF GROSS PATHOLOGY IN LIVERS (WHITE SPOTS) OR LUNGS (PNEUMONITIS) AT NECROPSY.* EXPERIMENT 4.

Pen & pig no.		Liver spots	Lungs pneumonitis	Ascarids present
17	35P	-	-	1
Fresh	36P	-	-	1
sludge	37P	+	-	-
	38P	+	+	-
	39P	+	-	4
	45P	+	-	2
18	F35	-	-	-
Control	F36	-	-	-
	F37	-	-	-
	F38	-	-	-
19	25P	+	-	1
Fresh	26P	-	-	-
sludge	27P	+	+	3
	28P	-	-	-
	29P	+	-	1
20	F25	-	-	-
Control	F26	-	-	-
	F27	-	-	-
	F28	-	-	-
	F29	-	-	-
21	15P	-	-	-
Nu-	16P	+	-	2
Earth	17P	-	-	-
from	18P	-	-	1
storage	19P	+	-	-
22	F15	-	-	-
Control	F16	-	-	-
	F17	-	-	-
	F18	-	-	-
	F19	-	-	-
23	05P	-	-	-
Fresh	06P	-	-	-
Nu-	07P	+	-	-
Earth	08P	-	-	-
	09P	-	-	-
24	F05	-	-	-
Control	F06	-	-	-
	F07	-	-	-

*Necropsied 10/26/79 and 10/30/79.

Figure 5. Location of pens showing physical appearance in field of alfalfa.

to Fulton County and then immediately spread in the pens, became infested with ascarids in the intestine (pens 17 and 19). None of the 17 surviving control pigs had any ascarids.

In none of the experiments indicated above, were we able to detect any kind of tissue parasites or parasites other than Ascaris sp. in the intestines, although examination of sludge indicated that ova of Trichuris sp. and Toxocara sp. were also present.

SUPPLEMENTAL STUDY NO. 1

Although this study was not specifically a part of the proposal, it is included for information purposes. The seven or eight pigs, placed in the pens, were exposed to embryonated or unembryonated human ascarid ova as described in the Materials and Methods Section of this report. After a 4 month contact period in the pens, all of the pigs were necropsied and were examined for parasites. Only 11 of the initial 15 pigs started on the trials survived to necropsy. The deaths of the four were attributed to the extremely high temperatures and humidities experienced in late August of 1979. None of the 11 survivors in pens that had been seeded with embryonated or unembryonated human Ascaris contained any worms in the

intestines at necropsy. This suggests that ascarids of human origin were not infective for swine when exposed in a natural environment. This raises some significant questions as to the concern for the presence of ascarid ova in sewage sludges. This area has been a subject of interest for those concerned with public health for some time. These results are certainly not conclusions, but they do raise a question as to the need for concern about the potential for transmission of Ascaris lumbricoides in sludge. Additional studies with human ascarids need to be conducted to further explore these findings.

SUPPLEMENTAL STUDY NO. 2

In this experiment liquid anerobically digested sludge containing 2.12% dry solids was aerated by bubbling air through it for 104 days and then was incorporated in the regular feed of young SPF pigs. Of the 3 pigs fed anerobically digested sludge, in regular pig feed over a period of 30 days, 1 of 3 contained ascarids in the intestine at necropsy. This indicates that Ascaris sp. ova, naturally present in anaerobically digested sewage sludge, are capable of embryonating and causing infections in susceptible pigs. Exposure to air is critical to the embryonation process; infection occurring only after that exposure. Each pig in this experiment received approximately 12 liters of liquid sludge which contained about 600 Ascaris sp. ova. It was not possible to recover all of the ova or to determine exactly how many embryonated.

SUPPLEMENTAL STUDY NO. 3

This study was conducted to determine the rate of natural embryonation of Ascaris ova of swine origin in the presence of soil or in the presence of soil treated with sludge or soil treated with Nu-earth. One week after placing the ova in soil variously exposed to sludge or Nu-earth, a few of the ova recovered were partially embryonated. After 7 weeks' exposure to soil or to soil treated with anerobically digested sludge in the form of liquid sludge or Nu-earth, an average of 71% of the ova recovered were embryonated and presumably were infective to swine (Table 10). The rate of embryonation increased until the sixth week when embryonation was presumed to have been complete. Some embryonation was detected at the end of the first and second weeks of exposure to the soil and sludge combination, but more embryonated ova were recovered after seven weeks, indicating a gradual embryonation of the ova as would normally be expected.

TABLE 10. RATES OF EMBRYONATION OF *ASCARIS* OVA, RECOVERED FROM UTERI OF ADULT FEMALE WORMS, (SWINE ORIGIN) EXPOSED TO SOIL, SOIL PLUS ANAEROBICALLY DIGESTED SLUDGE, OR SOIL PLUS NU-EARTH. OVA APPLIED TO PLOTS 7/17/79

Date of collection	Plot	No. of ova recovered	No. of ova embryonated to larval stages	Percent embryonation
7/24/79	1	0	0	0
	2	66	7	11
	3	7	0	0
	4	65	7	11
	5	68	4	6
	6	67	2	3
	7	0	0	-
	8	16	1	6
	avg			5
8/2/79	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	21	1	5
	5	100	2	2
	6	55	0	0
	7	10	0	0
	8	0	0	0
	avg			1
8/10/79	1	0	0	0
	2	67	19	28
	3	8	4	50
	4	0	0	0
	5	98	26	27
	6	70	31	44
	7	71	13	18
	8	17	0	0
	avg			24
8/20/79	1	0	0	0
	2	0	0	0
	3	0	0	0
	4	0	0	0
	5	84	67	80
	6	43	28	65
	7	16	7	44
	8	18	15	28
	avg			31
9/7/79	1	0	0	0
	2	11	6	55
	3	19	17	90
	4	5	1	83
	5	100	76	76
	6	100	80	80
	7	57	31	54
	8	86	53	71
	avg			62

Key to plots for Table 10.

1. Untreated control; no ova or sludge application.
2. 2.0×10^6 *Ascaris* ova applied to the surface of the ground.
3. 2.0×10^6 *Ascaris* ova seeded into 3 liters of sludge applied to the surface of the ground.
4. 2.0×10^6 *Ascaris* ova seeded into 6 liters of sludge applied to the surface of the ground.
5. 2.0×10^6 *Ascaris* ova seeded into 3 liters of sludge incorporated into the soil to a depth of 10 cm..
6. 2.0×10^6 *Ascaris* ova seeded into 6 liters of sludge incorporated into the soil to a depth of 10 cm..
7. 2.0×10^6 *Ascaris* ova seeded into 1 kg of Nu-Earth applied to the surface of the ground.
8. 2.0×10^6 *Ascaris* ova seeded into 2 kg of Nu-Earth applied to the surface of the ground.

COMMENTS ON *ASCARIS* STUDIES

In general, results of these studies indicate that ascarid ova, present in anaerobically digested sludge or in dehydrated sludge (Nu-earth), are infective or become infective for swine after exposure to air. This indicates that anerobically digested sludge, spread in the environment where pigs could come in contact with the sludge, could be a source of infection for the pigs. Although the number of worms initially would be low, if they produced eggs, they could seed the environment and future pigs grown at the same location would become more heavily infested.

Of particular significance was the finding that human ascarids failed to infest pigs that were specifically exposed, in a natural environment, to the ova. The study is suggestive, not conclusive, because of the lack of knowledge of the previous history of the worms supplying the ova used in this study. The ova came from a source originating in Colombia, South America. Although the ova were embryonated after exposure to 1% formalin and aeration, they apparently were not infective for swine in a normal environment on soil or on vegetation. The study raises an important question as to whether ascarid ova, regularly observed in sewage sludges, would be a threat to the human population. It is a widespread belief, at present, that ascarids in sewage sludge would indeed be a threat to humans. The failure of human ascarids to infest swine attests to a high degree of host specificity.

ANALYSIS OF TISSUES FOR HEAVY METALS

Because of the cost and time requirements, tissues from all four experiments were not analyzed. Tissues from experiment 1 and representative tissues from some groups of experiment 2 were analyzed and are reported.

Part of the overall purpose of this study was to ascertain the levels of heavy metals in tissues of pigs exposed to anaerobically digested sewage sludge or to the dehydrated sewage sludge, Nu-earth, produced by the Metropolitan Sanitary District of Greater Chicago. The study serves as a model for possible transmission of heavy metals from this type of environment to swine. As indicated, animals were grown in a natural environment by confinement in pens which had been variously treated with sludge. The pigs were allowed to reach maturity in this environment consuming regular commercially prepared feed and living the normal life of a pig. After approximately four months in the sludge-treated environment, the animals were necropsied and sample tissues were taken from the heart, liver, kidney, muscle, and bone, and chemical analyses were performed.

Experiment 1

Tables 11 through 17 show the results of analyses of tissues taken from pigs used in experiment 1. Tissues were analyzed for cadmium, chromium, copper, iron, nickel, lead, and zinc, and comparisons were made of the average levels present in the various tissues described. Exposure of the pigs to different quantities of sludge, according to the group to which they were assigned, resulted in uptake of some heavy metals in proportion to the level of exposure. For example, there was an increase in cadmium in the kidneys and livers of pigs exposed to increasing quantities of sludge (Table 11). In no instance during these studies which involved four large experiments in which pigs were exposed to sludge by contact and one small experiment in which sludge was fed directly to pigs, was there any indication of a pathological condition developing as a result of the exposure to the sludge or to the Nu-earth.

Tables 11 through 17 show the comparative levels of heavy metals in the different tissues of the animals in different groups. Table 18 is a summary of the heavy metals by groups of animals exposed to the different concentrations of sludge, Nu-earth, and soil in Experiment 1. Tables 24 and 25 show the results of analyses of liquid and dried sludge for heavy metals.

Experiment 2

Tables 19-22 show the concentrations of heavy metals in representative pigs necropsied in experiment 2. Table 19 shows the levels of heavy metals from control pigs. These may be compared with control pigs from experiment 1. Pigs were housed in pens in a slightly different area than those in experiment 1, but the results are similar. Table 20 shows the heavy metal levels in tissues from pigs exposed to sludge equivalent to 25 metric tons of sludge per hectare on the soil. Table 21 shows the heavy metals content in tissues of pigs having direct contact with Nu-earth (dehydrated sludge) for 4 months. Pigs lived in a confined area in which 18 metric tons of the material had been spread (see Figure 2). Pigs had maximum exposure by eating soil, wallowing in mud, and breathing dust as shown in Figures 6 and 7. Table 22 is a summary of the heavy metals in tissues from control pigs, pigs exposed to liquid sludge, and pigs exposed to Nu-earth.

TABLE 11. ANALYSIS FOR CADMIUM IN TISSUES OF PIGS EXPOSED TO DIFFERENT LEVELS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO SYSTEM. EXPERIMENT 1

(ppm, dry weight)

Group	Pig No.	Kidney	Liver	Muscle	Heart
GROUP 8	1	0.44	0.13	0.073	0.065
(control)	2	0.58	0.12	0.093	0.023
	4	0.70	0.14	0.072	0.100
	5	0.60	0.16	0.040	0.024
	Avg	0.58	0.14	0.069	0.053
GROUP 7	6	0.23	0.07	0.020	0.018
(control)	7	0.23	0.17	0.032	0.024
	8	0.55	0.08	0.016	0.030
	9	0.33	0.06	0.015	0.027
	Avg	0.34	0.10	0.021	0.025
GROUP 6	11	1.14	0.27	0.160	0.130
(22 DMT/Ha sludge)	12	1.22	0.21	0.055	0.024
	14	1.40	0.23	0.048	0.073
	15	0.77	0.14	0.057	0.064
	Avg	1.13	0.21	0.080	0.073
GROUP 5	16	1.40	0.50	0.047	0.016
(22 DMT/Ha sludge)	17	1.10	0.19	0.023	0.030
	18	1.01	0.21	0.035	0.030
	20	1.90	0.27	0.033	0.025
	Avg	1.35	0.29	0.034	0.025
GROUP 4	21	3.50	0.54	0.140	0.081
(65 DMT/Ha sludge)	22	2.10	0.39	0.140	0.130
	23	5.40	0.66	0.170	0.060
	25	4.30	0.73	0.140	0.079
	Avg	3.83	0.58	0.150	0.088
GROUP 3	26	2.00	0.35	0.200	0.800
(33 DMT/Ha sludge)	27	3.00	0.31	0.028	0.030
	28	1.90	0.28	0.027	0.029
	30	1.50	0.20	0.032	0.028
	Avg	2.10	0.29	0.072	0.222
GROUP 2	31	1.72	0.30	0.120	0.069
(22 DMT/Ha sludge)	33	1.30	0.21	0.090	0.040
	34	1.80	0.30	0.085	0.044
	35	1.20	0.13	0.042	0.056
	Avg	1.51	0.24	0.084	0.052
GROUP 1	36	0.20	0.06	0.017	0.014
(control)	37	0.63	0.11	0.017	0.040
	39	0.68	0.14	0.030	0.180
	40	0.38	0.10	0.045	0.053
	Avg	0.47	0.10	0.027	0.072
NB Standard Control (bovine liver)			0.27		

TABLE 12. ANALYSIS FOR CHROMIUM IN TISSUES OF PIGS EXPOSED TO DIFFERENT LEVELS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 1

(ppm, dry weight)

Group	Pig No.	Kidney	Liver	Muscle	Heart
GROUP 8	1			0.14	0.04
(control)	2			0.08	0.07
	4			0.12	0.09
	5			0.07	0.20
	Avg			0.10	0.10
GROUP 7	6			0.21	0.09
(control)	7			0.25	0.08
	8			0.06	0.05
	9			0.02	0.01
	Avg			0.14	0.06
GROUP 6	11			0.23	0.12
(22 DMT/Ha sludge)	12			0.13	0.04
	14			0.09	0.15
	15			0.15	0.11
	Avg			0.15	0.11
GROUP 5	16			0.06	0.06
(22 DMT/Ha sludge)	17			0.23	0.10
	18			0.15	0.09
	20			0.51	0.09
	Avg			0.24	0.08
GROUP 4	21			0.09	0.04
(65 DMT/Ha sludge)	22			0.14	0.14
	23			0.06	0.05
	25			0.06	0.03
	Avg			0.09	0.06
GROUP 3	26			0.12	0.07
(33 DMT/Ha sludge)	27			0.15	0.37
	28			0.15	0.05
	30			0.12	0.25
	Avg			0.14	0.18
GROUP 2	31			0.18	0.21
(22 DMT/Ha sludge)	33			0.09	0.06
	34			0.72	0.05
	35			0.08	0.09
	Avg			0.27	0.10
GROUP 1	36			0.28	0.14
	37			0.05	0.06
	39			0.06	0.09
	40			0.09	0.08
	Avg			0.12	0.09

Table 13. ANALYSIS FOR COPPER IN TISSUES OF PIGS EXPOSED TO DIFFERENT LEVELS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 1

(ppm, dry weight)

Group	Pig no.	Kidney	Liver	Muscle	Heart
GROUP 8	1	28	29	7.5	17
(control)	2	38	24	9.0	17
	4	32	16	6.1	17
	5	25	31	6.5	18
	Avg	31	25	7.3	17
GROUP 7	6	60	30	6.8	18
(control)	7	42	21	4.5	17
	8	62	25	7.8	16
	9	51	39	4.7	11
	Avg	54	29	6.0	16
GROUP 6	11	33	21	8.4	17
(22 DMT/Ha sludge)	12	25	23	6.5	17
	14	27	15	7.5	17
	15	30	20	3.8	14
	Avg	29	20	6.6	16
GROUP 5	16	25	18	5.5	16
(22 DMT/Ha sludge)	17	26	47	11.0	20
	18	40	34	3.5	18
	20	42	23	5.7	18
	Avg	33	31	6.4	18
GROUP 4	21	24	19	8.4	14
(65 DMT/Ha sludge)	22	44	31	7.8	17
	23	27	21	8.5	16
	25	35	22	8.8	17
	Avg	33	23	8.4	16
GROUP 3	26	26	29	11.8	22
(33 DMT/Ha sludge)	27	36	40	5.3	16
	28	30	51	6.2	16
	30	20	28	10.6	22
	Avg	28	37	8.5	19
GROUP 2	31	36	15	9.7	17
(22 DMT/Ha sludge)	33	36	25	9.4	16
	34	27	20	8.2	17
	35	23	16	3.5	12
	Avg	31	19	7.7	16
GROUP 1	36	27	19	8.8	17
(control)	37	42	17	7.0	16
	39	37	56	8.3	14
	40	23	16	3.3	11
	Avg	32	27	6.9	15
NB Standard Control (bovine liver)			188		

TABLE 14. ANALYSIS FOR IRON IN TISSUES OF PIGS EXPOSED TO DIFFERENT LEVELS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 1

(ppm, dry weight)

Group	Pig No.	Kidney	Liver	Muscle	Heart
GROUP 8	1	120	900	140	210
(control)	2	115	340	125	205
	4	165	500	90	195
	5	175	510	96	200
	Avg	144	562	113	202
GROUP 7	6	205	445	80	171
(control)	7	130	670	70	190
	8	165	520	115	205
	9	127	266	50	105
	Avg	157	475	79	168
GROUP 6	11	140	790	165	200
(22 DMT/Ha sludge)	12	180	440	70	200
	14	140	460	105	210
	15	105	350	60	150
	Avg	141	510	100	190
GROUP 5	16	105	1220	75	190
(22 DMT/Ha sludge)	17	109	613	105	152
	18	140	620	100	250
	20	130	607	90	196
	Avg	121	765	92	197
GROUP 4	21	196	403	110	134
(65 DMT/Ha sludge)	22	135	730	115	180
	23	175	610	130	190
	25	180	600	120	215
	Avg	172	586	119	180
GROUP 3	26	129	522	107	151
(33 DMT/Ha sludge)	27	164	581	79	164
	28	150	560	90	195
	30	134	534	87	165
	Avg	144	549	91	169
GROUP 2	31	200	625	125	215
(22 DMT/Ha sludge)	33	165	500	110	170
	34	190	675	140	195
	35	130	450	75	140
	Avg	171	562	112	180
GROUP 1	36	220	640	110	200
(control)	37	180	490	105	200
	39	200	460	110	175
	40	112	410	50	125
	Avg	178	500	94	175

TABLE 15. ANALYSIS FOR NICKEL IN TISSUES OF PIGS EXPOSED TO DIFFERENT LEVELS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 1

(ppm, dry weight)

Group	Pig No.	Kidney	Liver	Muscle	Heart
GROUP 8	1			0.2	
(control)	2			0.6	
	4			0.4	
	5			0.2	
	Avg			0.4	
GROUP 7	6			-	
(control)	7			0.2	
	8			0.5	
	9			-	
	Avg			0.4	
GROUP 6	11			0.4	
(22 DMT/Ha sludge)	12			0.3	
	14			0.4	
	15			0.2	
	Avg			0.3	
GROUP 5	16			0.3	
(22 DMT/Ha sludge)	17			-	
	18			0.2	
	20			-	
	Avg			0.3	
GROUP 4	21			-	
(65 DMT/Ha sludge)	22			0.7	
	23			0.4	
	25			0.6	
	Avg			0.6	
GROUP 3	26			-	
(33 DMT/Ha sludge)	27			-	
	28			0.3	
	30			-	
	Avg			0.3	
GROUP 2	31			1.0	
(22 DMT/Ha sludge)	33			0.7	
	34			0.4	
	35			0.2	
	Avg			0.6	
GROUP 1	36			0.4	
(control)	37			0.7	
	39			0.5	
	40			0.2	
	Avg			0.5	

TABLE 16. ANALYSIS FOR LEAD IN TISSUES OF PIGS EXPOSED TO DIFFERENT LEVELS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 1

(ppm, dry weight)

Group	Pig No.	Kidney	Liver	Muscle	Heart
GROUP 8 (control)	1	0.18	0.28	0.12	0.18
	2	0.21	0.29	0.18	0.19
	4	0.36	0.44	0.39	0.21
	5	0.24	0.25	0.16	0.22
	Avg	0.25	0.32	0.22	0.20
GROUP 7 (control)	6	0.34	0.29	0.12	0.18
	7	0.27	0.70	0.13	0.22
	8	0.27	0.25	0.16	0.19
	9	0.28	0.20	0.09	0.10
	Avg	0.29	0.36	0.12	0.17
GROUP 6 (22 DMT/Ha sludge)	11	0.29	0.34	0.42	0.24
	12	3.40	0.30	0.13	0.16
	14	0.26	0.26	0.33	0.20
	15	0.22	0.21	0.36	0.18
	Avg	1.05	0.28	0.31	0.20
GROUP 5 (22 DMT/Ha sludge)	16	0.24	2.20	0.16	0.19
	17	0.25	0.30	0.18	0.18
	18	0.24	0.27	0.12	0.22
	20	0.26	0.34	0.14	0.18
	Avg	0.25	0.78	0.15	0.19
GROUP 4 (65 DMT/Ha sludge)	21	0.25	0.25	0.15	0.15
	22	0.41	0.32	0.34	0.21
	23	0.32	0.36	0.15	0.17
	25	0.29	0.30	0.15	0.19
	Avg	0.32	0.31	0.20	0.38
GROUP 3 (33 DMT/Ha sludge)	26	0.29	0.35	0.17	0.17
	27	0.30	0.36	0.14	0.19
	28	0.27	0.47	0.20	0.20
	30	0.30	0.28	0.16	0.18
	Avg	0.29	0.36	0.17	0.18
GROUP 2 (22 DMT/Ha sludge)	31	0.31	0.32	0.38	0.21
	33	0.28	0.38	0.33	0.18
	34	0.30	0.73	0.38	0.21
	35	0.28	0.18	0.25	0.13
	Avg	0.29	0.40	0.34	0.18
GROUP 1 (control)	36	0.61	0.31	0.38	0.21
	37	0.29	0.36	0.20	0.18
	39	0.36	0.29	0.37	0.18
	40	0.20	0.21	0.23	0.12
	Avg	0.37	0.29	0.30	0.17
NB Standard Control (bovine liver)			0.35		

TABLE 17. ANALYSIS FOR ZINC IN TISSUES OF PIGS EXPOSED TO DIFFERENT LEVELS OF ANAEROBICALLY DIGESTED SEWAGE SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 1

(ppm, dry weight)

Group	Pig No.	Kidney	Liver	Muscle	Heart
GROUP 8	1	110	270	105	81
(control)	2	137	365	160	75
	4	132	227	-	85
	5	120	500	131	85
	Avg	125	341	132	82
GROUP 7	6	121	191	83	68
(control)	7	131	325	88	85
	8	188	225	156	82
	9	178	165	65	39
	Avg	154	226	98	68
GROUP 6	11	122	281	-	85
(22 DMT/Ha sludge)	12	117	239	88	75
	14	122	230	-	85
	15	117	168	-	38
	Avg	120	230	88	71
GROUP 5	16	118	537	100	80
(22 DMT/Ha sludge)	17	102	210	148	76
	18	144	338	90	90
	20	137	320	98	62
	Avg	125	351	109	77
GROUP 4	21	110	138	122	53
(65 DMT/Ha sludge)	22	146	230	-	84
	23	127	214	148	75
	25	127	259	53	84
	Avg	128	210	108	74
GROUP 3	26	111	260	140	52
(33 DMT/Ha sludge)	27	106	180	93	61
	28	144	412	131	82
	30	108	139	112	65
	Avg	117	248	119	65
GROUP 2	31	156	235	-	81
(22 DMT/Ha sludge)	33	132	224	-	74
	34	132	224	-	81
	35	142	120	-	55
	Avg	141	201	-	73
GROUP 1	36	117	224	-	89
(control)	37	137	185	145	76
	39	151	240	-	66
	40	119	173	-	52
	Avg	131	206	145	71
NB Standard Control (bovine liver)			131		

TABLE 18. SUMMARY OF HEAVY METALS IN SWINE TISSUES. EXPERIMENT 1

(ppm, dry weight)

Metal – Group	No. of Pigs	Kidney	Liver	Muscle	Heart
Cadmium					
22 DMT/Ha sludge	12	1.33	0.25	0.07	0.05
33 DMT/Ha sludge	4	2.10	0.29	0.09	0.22
65 DMT/Ha sludge	4	3.83	0.58	0.15	0.09
Control	12	0.47	0.15	0.05	0.05
NB Standard Control	-	-	0.27	-	-
Chromium					
22 DMT/Ha sludge	12			0.22	0.10
33 DMT/Ha sludge	4			0.14	0.18
65 DMT/Ha sludge	4			0.09	0.06
Control	12			0.12	0.08
Copper					
22 DMT/Ha sludge	12	31	23	6.9	16.7
33 DMT/Ha sludge	4	28	24	8.5	19.0
65 DMT/Ha sludge	4	33	37	8.4	16.0
Control	12	39	27	6.7	15.0
NB Standard Control	-	-	188	-	-
Iron					
22 DMT/Ha sludge	12	144	612	101	189
33 DMT/Ha sludge	4	144	549	91	169
65 DMT/Ha sludge	4	172	586	119	180
Control	12	160	512	95	182
Nickel					
22 DMT/Ha sludge	12			0.4	
33 DMT/Ha sludge	4			0.3	
65 DMT/Ha sludge	4			0.6	
Control	12			0.4	
Lead					
22 DMT/Ha sludge	12	0.53	0.49	0.27	0.19
33 DMT/Ha sludge	4	0.29	0.36	0.17	0.18
65 DMT/Ha sludge	4	0.32	0.20	0.20	0.38
Control	12	0.30	0.32	0.21	0.18
NB Standard Control	-	-	0.35	-	-
Zinc					
22 DMT/Ha sludge	12	129	261	99	74
33 DMT/Ha sludge	4	117	248	119	65
65 DMT/Ha sludge	4	128	210	108	74
Control	12	137	258	125	74
NB Standard Control	-	-	131	-	-

TABLE 19. ANALYSIS FOR HEAVY METALS IN TISSUES OF CONTROL PIGS NOT DIRECTLY EXPOSED TO SLUDGE. EXPERIMENT 2

(ppm, dry weight)

Exposure	Pig No.	Kidney	Liver	Muscle	Heart	Bone
Cadmium						
Control	P35F	0.65	1.7	0.084	0.053	0.029
No sludge	P46F	0.37	NA	0.054	0.011	0.026
	Avg	0.51	1.7	0.069	0.032	0.028
Copper						
Control	P35F	22.0	15.0	8.6	18.0	3.3
No sludge	P46F	32.0	28.0	9.5	15.4	3.7
	Avg	27.0	22.0	9.1	16.7	3.5
Iron						
Control	P35F	252	299	102	162	33
No sludge	P46F	160	381	143	175	54
	Avg	206	340	123	169	44
Lead						
Control	P35F	0.28	0.35	0.25	0.28	0.73
No sludge	P46F	0.42	0.34	0.29	0.27	0.52
	Avg	0.35	0.35	0.27	0.28	0.63
Zinc						
Control	P35F	131	382	140	82	80
No sludge	P46F	127	247	182	83	115
	Avg	129	315	161	83	98

TABLE 20. ANALYSIS FOR HEAVY METALS IN TISSUES OF PIGS EXPOSED TO 25 DRY METRIC TONS OF ANAEROBICALLY DIGESTED SLUDGE ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 2

(ppm, dry weight)

Exposure	Pig No.	Kidney	Liver	Muscle	Heart	Bone
Cadmium						
25 DMT/Ha sludge	K15	3.6	0.23	0.058	0.044	0.011
	K18	3.2	0.18	0.036	0.032	0.013
	K27	3.8	0.41	0.061	0.040	0.019
	K35	9.0	0.70	0.064	0.061	0.019
	K38	9.3	1.40	0.036	0.037	0.026
	K46	2.3	0.30	0.110	0.048	0.015
	Avg	5.2	0.54	0.061	0.044	0.0172
Copper						
25 DMT/Ha sludge	K15	52.0	21.0	5.2	17.5	3.5
	K18	51.0	26.0	18.0	18.3	2.9
	K27	34.0	23.0	9.1	17.8	3.3
	K35	26.0	33.0	9.1	19.5	3.4
	K38	28.0	25.0	9.3	18.3	3.1
	K46	37.0	26.0	11.2	19.5	3.4
	Avg	38.0	26.0	10.3	18.5	3.3
Iron						
25 DMT/Ha sludge	K15	168.0	502.0	69.0	181.0	59.0
	K18	159.0	297.0	142.0	172.0	50.0
	K27	204.0	323.0	121.0	188.0	39.0
	K35	195.0	502.0	131.0	219.0	65.0
	K38	166.0	493.0	120.0	160.0	33.0
	K46	163.0	569.0	140.0	170.0	19.0
	Avg	176.0	448.0	121.0	182.0	44.0
Lead						
25 DMT/Ha sludge	K15	0.38	0.44	0.27	0.33	0.88
	K18	0.35	0.37	0.40	0.28	0.85
	K27	0.30	0.42	0.31	0.29	0.90
	K35	0.43	0.47	0.27	0.30	1.4
	K38	0.32	0.33	0.31	0.32	0.075
	K46	0.28	0.32	0.38	0.34	0.05
	Avg	0.34	0.39	0.32	0.31	0.69
Zinc						
25 DMT/Ha sludge	K15	163.0	328.0	75.0	74.0	116.0
	K18	158.0	212.0	155.0	60.0	85.0
	K27	152.0	315.0	166.0	75.0	104.0
	K35	123.0	232.0	167.0	91.0	101.0
	K38	136.0	269.0	158.0	80.0	112.0
	K46	112.0	250.0	168.0	74.0	192.0
	Avg	141.0	268.0	148.0	76.0	118.0

TABLE 21. ANALYSIS FOR HEAVY METALS IN TISSUES OF PIGS CONFINED TO PENS FILLED WITH DRIED ANAEROBICALLY DIGESTED SLUDGE (NU-EARTH) ORIGINATING FROM THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. EXPERIMENT 2

(ppm--dry weight)

Exposure	Pig #	Kidney	Liver	Muscle	Heart	Bone
Cadmium						
Nu-earth*	P15F	9.8	1.8	0.11	0.11	0.044
	P16F	11.0	1.5	0.025	0.035	0.046
	P17F	11.5	1.7	0.055	0.044	0.037
	P18F	6.9	1.1	0.036	0.051	0.031
	Avg	9.8	1.5	0.056	0.060	0.039
Copper						
Nu-earth*	P15F	33.0	32.0	8.5	17.7	5.0
	P16F	41.0	23.0	5.4	13.1	3.5
	P17F	38.0	58.0	8.0	17.9	3.5
	P18F	64.0	17.3	4.9	22.0	3.2
	Avg	44.0	32.6	6.7	17.7	3.8
Iron						
Nu-earth*	P15F	214.0	525.0	87.0	151.0	38.0
	P16F	241.0	531.0	60.0	110.0	59.0
	P17F	182.0	412.0	90.0	155.0	43.0
	P18F	153.0	343.0	54.0	190.0	27.0
	Avg	198.0	453.0	73.0	152.0	42.0
Lead						
Nu-earth*	P15F	0.26	0.32	0.25	0.26	0.79
	P16F	0.36	0.36	0.20	0.22	0.79
	P17F	0.82	0.93	0.24	0.27	4.20
	P18F	0.30	0.28	0.16	0.29	1.30
	Avg	0.44	0.47	0.21	0.26	1.77
Zinc						
Nu-earth*	P15F	113.0	201.0	150.0	83.0	108.0
	P16F	171.0	278.0	94.0	64.0	118.0
	P17F	136.0	297.0	145.0	82.0	110.0
	P18F	140.0	368.0	64.0	79.0	150.0
	Avg	140.0	286.0	113.0	77.0	122.0

*1134 DMT/Ha

TABLE 22. SUMMARY OF HEAVY METALS IN TISSUES OF PIGS LIVING IN PENS FOR WHICH SOIL WAS TREATED OR NOT TREATED WITH SLUDGE. EXPERIMENT 2

(ppm, dry weight)

Metal and Group	No. of Pigs	Kidney	Liver	Muscle	Heart	Bone
Cadmium						
Control	2	0.51	1.70	0.069	0.032	0.028
25 DMT/Ha	6	5.20	0.54	0.061	0.044	0.017
Nu-earth	4	9.80	1.50	0.056	0.060	0.039
Copper						
Control	2	27	22	9.1	16.7	3.5
25 DMT/Ha	6	38	26	10.3	18.5	3.3
Nu-earth	4	44	33	6.7	17.7	3.8
Iron						
Control	2	206	340	123	169	44
25 DMT/Ha	6	176	448	121	182	44
Nu-earth	4	198	453	73	152	42
Lead						
Control	2	0.35	0.35	0.27	0.28	0.63
25 DMT/Ha	6	0.34	0.39	0.32	0.31	0.69
Nu-earth	4	0.44	0.47	0.21	0.26	1.77
Zinc						
Control	2	129	315	161	83	98
25 DMT/Ha	6	141	268	148	76	118
Nu-earth	4	140	286	113	77	122

Figure 6. Typical exposure of pigs to soil treated with sludge or Nu-earth where heavy rains saturated pens.

Figure 7. Typical dusty dry pen when no rain had occurred for several weeks. Pigs breathed dust stirred up by wind and their own activity. A portion of dust came from sludge and Nu-earth.

STATISTICAL ANALYSIS OF HEAVY METALS IN TISSUES

A statistical comparison of heavy metals in most tissues was made. Kidney and liver tissues show the greatest concentrations of heavy metals such as cadmium, but heart, muscle, and bone were also compared where there appeared to be a difference in heavy metals.

A comparison of cadmium levels, in the kidneys of pigs in experiments 1 and 2, between controls and all levels of exposure to sludge, indicated a significant difference at the 99% confidence level suggesting that the levels of cadmium in the kidneys were directly attributable to the exposure of the pigs to the sludge. Comparisons of cadmium levels in liver tissue showed a similar confidence interval at the highest level of application of sludge to a pen (65 DMT/Ha) in experiment 1, but there was no significant difference in tissues from pigs in experiment 2. However, there were only 12 animals examined in experiment 2, and this may have influenced the results.

There was no significant difference in cadmium content of muscle and heart tissue from experimental animals when compared to the same tissues from control animals in either experiment. There were no significant differences in the levels of chromium, iron, nickel, or zinc in tissues of control and experimental animals.

Approximately twice as much lead was present in the bones of pigs that had lived in close association with Nu-earth or with the 65 DMT/Ha concentration of sludges in soil for four months when compared to the bones from control pigs. However, there was no statistical difference in the lead content from tissues in these groups of animals.

A striking difference was observed in the copper content of the livers of the pigs from both experiments when compared to the National Bureau of Standards bovine liver. The copper content of pig liver in the present studies was only about 1/7 that of copper in bovine liver samples obtained from the National Bureau of Standards. It has been pointed out by Swenson (21) and others that there are wide differences in copper concentration within and between species. The copper content of liver from pigs, man, dog, cats, and domestic fowls ranges from 10-50 ppm on a dry weight basis. A species difference appears to explain the variation in results when our studies are compared with the National Bureau of Standards bovine liver standard.

Concentrations of zinc in livers were much higher in our studies than in bovine liver from the National Bureau of Standards. The zinc content in pig liver was twice as high as the bovine liver. There were no statistical differences between groups in our studies however. Apparently zinc content is highly variable and is influenced by a number of factors including the presence and the level of cadmium (21).

HEAVY METALS IN SOILS, SLUDGE, AND NU-EARTH

Tables 23 and 23a shows the average heavy metals content in soils from various representative control pens not treated with sludge or from pens treated with different quantities of sludge or Nu-earth.

Table 24 shows the total chemical analysis of liquid sludge placed in the pens in July 1978. These samples are typical of the sludges used to treat the soil of the pens in which the pigs were confined during these experiments. Applications of sludge to pens, indicated in the text and recorded as quantity per hectare, were calculated from average analyses.

Nu-earth, the dehydrated sludge prepared by the Metropolitan Sanitary District of Greater Chicago and used in this study, had relatively high levels of some heavy metals as is shown in table 25. The figures shown are average values taken from several samples of the material. Data furnished by the Metropolitan Sanitary District of Greater Chicago.

Table 26 shows the heavy metals content of commercial pig feed (Farm Services, Fiatt, Illinois) and represents a typical pig feed used in the area and can be considered a standard control feed.

COMMENTS ABOUT HEAVY METALS

Although we were unable to analyze all of the tissues collected for heavy metals, those examined appear to be a fair sampling of what one could expect to find in tissues of animals raised under similar conditions. The data obtained show that there is a significant increase in heavy metals, such as cadmium, when swine are exposed to sewage sludges that contain levels of heavy metals greater than that found in the natural environment. Accumulation of cadmium in the kidney and liver is most pronounced. Studies have shown that about four or five percent of the cadmium ingested is stored in the body. The other heavy metals, observed in this study, appear to be more or less actively metabolized and balanced in the body. Lead, in the bones, may be an exception.

The main question then becomes, "What is the real practical effect of exposure of animals, such as swine, to sewage sludges?" One can only give a speculative answer based upon the data, combined with observations and experience. Obviously, the quality of the sludge and the length of exposure of the animals to it would determine the quantities of heavy metals accumulated in the tissues. Dried or otherwise concentrated sludges, contain the greatest quantities of both organic and inorganic chemicals. It would appear that exposure to sewage sludges, similar to those used in these studies, would not cause any immediate effect upon the health of swine, or probably other animals. Continual maximal exposure (living in pens consisting of dried sludge) to sludges, such as Nu-earth, for a calculated period of 22+ years could possibly cause accumulation of enough cadmium in the kidneys to begin causing physiological malfunction. Since the normal life span of a typical "feeder" pig is much less than

TABLE 23. RESULTS OF CHEMICAL ANALYSES OF SOILS, TREATED WITH ANAEROBICALLY DIGESTED SLUDGE OR NOT TREATED, FOR HEAVY METALS.* EXPERIMENTS 1, 2, 3, AND 4

(ppm--dry weight)

Soil from pen treated/nontreated	Zn	Cu	Cd	Cr	Ni	Pb	Fe
Pen #1 (control)	374	13	0.37	7	27	46.0	17000
Pen #1 (control)	38	4	0.40	9	14	4.0	8000
Pen #7 (control)	81	-	0.50	29	48	2.1	13000
Pen #8 (control)	128	-	0.70	9	17	7.5	10000
Pen #9 (control)	96	13	0.22	16	36	26.0	18000
Average	143	10	0.44	14	28	17.0	13200
Pen #2 (22 MT) x 1	140	13	1.50	19	17	52.0	20000
Pen #5 (22 MT) x 1	120	38	5.90	54	26	55.0	15000
Pen #6 (22 MT) x 1	169	-	3.90	30	47	6.5	9200
Pen #13 (25 MT) x 2, 3	200	54	8.70	93	52	40.0	6400
Pen #13 (25 MT) x 2, 3	270	68	16.00	130	41	50.0	19000
Pen #16 (25 MT) x 2, 3	170	48	10.00	92	30	41.0	8000
Average	178	44	7.70	70	36	41.0	12933
Pen #3 (33 MT) x 1	142	39	6.80	84	46	27.0	20000
Pen #4 (65 MT) x 1	560	145	37.00	340	70	105.0	19000
Pen #12 (Nu-earth) x 2, 3	1800	300	90	300	62	800	15000
Pen #12 (Nu-earth) x 2, 3	3300	1100	260	760	190	460	8900
Pen # 5 (Nu-earth) x 4	3200	1000	270	1000	330	720	8400
Pen #7 (Fresh Nu-earth) x 4	3400	1400	280	970	250	1100	9500
Pen #7 (Fresh Nu-earth) x 4	3400	1200	280	1100	410	680	7400
Average	3020	1000	236	826	248	752	9840

*Twice listed pens represent samples taken from different areas of pens.

TABLE 23a. AVERAGES OF CHEMICAL ANALYSES OF SOIL SAMPLES FOR HEAVY METALS. PIGS WERE CONFINED IN PENS TO WHICH SLUDGE WAS ADDED IN THE EQUIVALENT DRY METRIC TONS PER HECTARE SHOWN. EXPERIMENTS 1, 2, 3, and 4

Soil from pen treated/nontreated	ppm--dry weight						
	Zn	Cu	Cd	Cr	Ni	Pb	Fe
Control	143	10	0.44	14	28	17	13200
22-25 DMT/Ha	178	44	7.70	70	36	41	12933
33 DMT/Ha	142	39	6.80	84	46	27	20000
65 DMT/Ha	560	145	37.00	340	70	105	19000
1134 DMT/Ha (Nu-earth)	3020	1000	236.00	826	248	752	9840

that (6 to 7 months), it would appear that significant effects upon the health of the pig would be nil.

If man was to ingest muscle tissues from a pig living in a dried sludge environment, such as was used in these studies, the amount of cadmium received would be only slightly higher than that obtained from muscle tissues from pigs not exposed to sludge. The total cadmium received in a pork chop, for example, from an animal continuously exposed to dried sludge for four months, would be roughly equivalent to smoking two or three cigarettes.

POLYCHLORINATED BIPHENYLS

Table 27 shows the comparative levels of the organic compounds (PCB's, heptachlor epoxide, and dieldrin) present in visceral fat taken from the abdominal cavity of pigs in experiment 4. It is known that fat accumulates PCB's and hydrocarbons, and the concentrations are much higher than would be present in other body tissues. It is not known whether rendered fat would contain the same quantity of PCB's or hydrocarbons as was present in the fat taken directly from the viscera.

The results of the chemical analyses of fat samples indicates that pigs living in an environment of continuous exposure to anaerobically digested sludge from the Metropolitan Sanitary District of Greater Chicago are not significantly different from control pigs. As a matter of fact, fat from pigs continuously exposed to Nu-earth (presumed to have the highest content of these organic compounds) had mean levels of organics lower than the fat from control pigs. Levels of organics in the Nu-earth are

TABLE 24. ANALYSIS OF LIQUID ANAEROBICALLY DIGESTED SLUDGE APPLIED TO PENS IN WHICH PIGS WERE CONFINED. ORIGINATED FROM LAGOON STORAGE. DATA FURNISHED BY THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO.

pH		7.6
Total solids		4.5%
Total volume solids		48.3%
Total P	mg/L	1526.7
N-Kjeldahl	mg/L	2669.0
$N\text{-}NH_3$	mg/L	1072.0
Alk. as $CaCO_3$	mg/L	3407.0
Cl	mg/L	400.0
E.C.	ummhos/cm	2967.0
Fe	mg/L	2297.0
Zn	mg/L	188.0
Cu	mg/L	89.8
Ni	mg/L	20.0
Mn	mg/L	23.1
K	mg/L	170.0
Na	mg/L	83.0
Mg	mg/L	433.0
Ca	mg/L	1293.0
Pb	mg/L	42.2
Cr	mg/L	283.0
Cd	mg/L	13.7
Al	mg/L	133.8
Hg	ug/L	514.0

not available, but analysis of liquid sludge from the MSDGC system for the years 1975-78 show a mean of 4.967 PPM, for PCB's, less than 0.10 PPB heptachlor, and 59 PPB for dieldrin according to U.S. Geological Survey analyses (22). Pigs living in this environment would have a maximum natural exposure which would probably not be duplicated under normal husbandry conditions.

These results indicate that these organics probably do not constitute a threat to animal or human life when dispersed in the environment under conditions similar to those described in this report.

TABLE 25. ANALYSIS OF NU-EARTH STORED AT THE HARLEM AVENUE SITE OF THE METROPOLITAN SANITARY DISTRICT OF GREATER CHICAGO. 1976

	Average mg/kg
Zinc	3598
Cadmium	191
Copper	1711
Chromium	2219
Iron	26575
Nickel	323
Lead	1032
Potassium	1155
Sodium	609
Calcium	32054
Magnesium	11863
Manganese	277
Aluminum	8447
Mercury (ug/kg)	7756

TABLE 26. HEAVY METALS CONTENT OF COMMERCIAL PIG FEED FED TO PIGS

(ppm--dry weight)

Metal	Amount in feed
Cd	3
Cu	7
Fe	108
Ni	2
Pb	26
Zn	169

TABLE 27. ANALYSIS FOR POLYCHLORINATED BIPHENYLS AND CHLORINATED HYDROCARBONS IN PIG VISCERAL FAT. EXPERIMENT 4

(ppm--wet weight)

Animal no. and exposure	PCB's	Heptachlor epoxide	Dieldrin
Control:			
F05	0.0901	0.0038	0.0910
F06	0.1573	0.1101	0.1017
F07	0.2373	0.2364	0.1091
F15	0.1000	0.0044	0.0028
F16	0.1211	0.0998	0.1003
F17	0.1572	0.1555	0.2400
F18	-	-	0.0009
F19	-	0.0020	0.0017
F25	-	0.0037	0.0020
F26	0.2322	0.0021	0.0009
F27	-	0.0014	0.0014
F28	-	0.0759	0.0737
F29	0.1874	0.0890	0.0012
F35	0.1201	0.0013	0.0020
F36	0.1740	0.0818	0.0012
F37	-	0.0805	0.1364
F38	-	-	0.0011
Avg	0.1577	0.0632	0.0510
1000 Gallon Sludge:			
25P	0.2056	0.0043	0.0012
26P	0.1306	0.0030	0.0016
27P	0.1003	0.0071	0.0083
29P	-	-	-
35P	0.1000	0.0203	0.0300
37P	0.1330	0.0015	0.0017
38P	-	-	0.0007
39P	0.1007	0.0001	0.0001
45P	0.0079	0.0003	0.0007
Avg	0.1112	0.0052	0.0055
Nu-Earth:			
05P	0.1020	-	0.0184
06P	0.1011	0.0013	0.0010
07P	0.0138	-	0.0018
08P	0.1258	0.0009	0.0006
09P	0.3153	0.0014	0.0011
15P	0.0125	0.0008	0.0012
16P	-	0.0023	0.0025
17P	0.1572	-	0.0005
18P	0.1301	0.0022	0.0022
19P	-	-	-
Avg	0.1197	0.0015	0.0033

SUMMARY

In the work reported herein, the objectives were to determine the possibility of transmission of one pathogen (Ascaris lumbricoides suum) from soil treated with sludge to a susceptible host (a pig). Since this organism is almost identical to the one that occurs in humans, it was assumed that information obtained could be extrapolated to represent a potential source of infection in humans. Likewise, pigs living in an environment variously treated with sewage sludges containing heavy metals and organic compounds could serve as models to determine the potential for transmission of toxic chemicals to humans.

A total of 178 SPF pigs were used to study whether anaerobically digested sludge, added to soil at different levels of concentration, could be a potential source of infestation with the nematode Ascaris lumbricoides suum. The soil in 9.8 x 14.6 M pens was treated with liquid anaerobically digested sludge (22 to 65 dry metric tons per hectare) or with dried sludge (Nu-Earth). Specific Pathogen Free pigs were confined to the pens in control and experimental groups for 4 months. In two experiments, eight young pigs were necropsied 2-3 weeks after introduction into the pens, and lung and liver tissues were baermannized to recover migrating stages of the worms. Larvae were recovered from the lungs of 2 of 16 necropsied.

Of 134 pigs, 43 contained ascarid worms in the intestines, including four from the control pens. The latter were the result of accidental contamination. These results represent an infection rate of 28% in the experimental groups and 3% in the control groups. Six of 11 pigs housed in the pens treated with Nu-Earth were infested.

An attempt was made to determine the rate of transmission of the human ascarid, obtained from a source in Columbia, South America, to pigs. Fifteen SPF pigs were confined in two sludge-free pens. Embryonated or unembryonated ova, in water, were sprayed onto the soil or vegetation at the rate of 572,000 ova in each of the two pens. The ova were extracted from the uteri of adult female worms, and those that were embryonated were embryonated in 1% formalin with continuous aeration for 30 days. At necropsy, 4 months after being placed in the pens, no worms were found in either group of pigs.

In another experiment, three pigs were fed anaerobically digested sludge in which there were naturally occurring ascarid ova. The anaerobically digested sludge was aerated by bubbling air through it for 104 days. It was then fed to each of the three pigs during a 30-day period. Forty-seven days after the end of the feeding period, the pigs were necropsied, and worms were found in the intestine of one pig.

An experiment was conducted to determine the rate of embryonation of ascarid ova (removed from the uteri of gravid female worms) in the presence of untreated soil, soil treated with liquid anaerobically digested sludge, or Nu-Earth. At the end of six weeks, exposure to the

soil, to which sludge had been added, an average 71% of the ova had embryonated and were presumed to be infective.

In two of the experiments, tissues were taken from the pigs at necropsy for chemical analysis for heavy metals and organic compounds. Diaphragm, muscle, heart, liver, kidney, and bone were selected for analysis for heavy metals, and visceral fat was selected for analysis for the organic compounds.

The only heavy metal showing significant accumulation was that of cadmium in the kidney and liver. The levels of accumulation in tissues show that some heavy metals, present in sludges, may be accumulated in the tissues at levels dependent upon the level of exposure, i.e., animals exposed to greater concentrations of sludge had greater concentrations of some heavy metals in the tissues. Pigs exposed to dried sludge (Nu-Earth) accumulated the greatest quantities of heavy metals.

The results of chemical analysis for organic compounds (PCB's, Dieldrin, Heptachlor) showed there was no difference between experimental and control animals in terms of accumulation. The levels of these compounds were not accumulated in the animal tissues at concentrations greater than is normally found in the environment.

REFERENCES

1. Dalton, F. E., and R. R. Murphy. Land Reclamation. A Recycle Which Completely Solves the Sewage Solids Problem. Ann. Conf. Water Poll. Cont. Fed., Atlanta, Ga., October 9, 1972.

2. Peterson, J. R., C. Lue-Hing, and D. R. Zenz. Chemical and Biological Quality of Municipal Sludges. Symposium of Recycling Treated Murine Waste Water, Pennsylvania State University, August 22, 1972.

3. Purves, D. Consequences of Trace-Element Contamination of Soils. Environ. Poll., 3:17-24, 1972.

4. Dotson, G. K. Constraints to Spreading Sewage Sludge on Cropland. News of Environmental Research in Cincinnati, May 31, 1973.

5. Jolley, W. R., and P. R. Fitzgerald. Sludge In the Pasture (?). Ill. Res., 18:10-11, 1976.

6. Abbot, A. L. Grazing of Cattle on Sewage Farms and Disposal Works. Pub. Health (S. Africa), p. 76-88. Abstr. in Sewage Works Journal 21:185-186, 1948.

7. Burge, W. D. Health Aspects of Applying Sewage Wastes to Land. North Central Regional Conference Workshop, Kellog Center, Michigan State University, September 23-24, 1974.

8. Davis, D. D., R. Dulbecco, H. E. Elsen, H. S. Ginsberg, W. B. Wood, and M. McCarty. Microbiology. Harper and Row, Hagerstown, Maryland, 1973, 1562 pp.

9. Chobanov, R. E., and N. G. Dashkova. Comparative Evaluation of the Effectiveness of Modern Sanitary Helminthological Methods of Investigating Soil. Gig. Sanit., 1:77-80, 1973.

10. Rowe, D. R. Anaerobic Sludge Digestion Mesophilic-Thermophilic. Water and Sewage Works, 118:74-76, 1971.

11. Fitzgerald, P. R. Potential Impact on the Public Health Due to Parasites in Soil/Sludge Systems. In: Proceedings of the 8th National Congress on Municipal Sludge Management, Miami Beach, Florida, 1979. pp. 214-221.

12. Maly, J., and H. Fadries. Influence of Temperature on Anaerobic Digestion. J. Water Poll. Cont. Fed., 43:641-650, 1971.

13. Blood, D. C. and J. A. Henderson. Veterinary Medicine. 3rd ed., Williams and Wilkins Co., Baltimore, 1968.

14. Curds, C. R. and A. Cockburn. Protozoa in Biological Sewage Treatment Processes. Water Pol. Abst., 44:248, 1971.

15. Hayes, B. D. Is There a Potential for Parasitic Disease Transmission from Land Application of Sewage Effluents and Sludges. J. Environ. Health, 39:424-426, 1977.

16. Fitzgerald, P. R., and R. F. Ashley. Differential Survival of Ascaris Ova in Wastewater Sludge. J. WPCF, 49:1722-1724, 1977.

17. Mueller, W. D. Health Aspects of Applying Sewage Wastes to Land. North Central Regional Conference Workshop. Kellog Center, Michigan State University, September 23-24, 1974.

18. Woodruff, A. W. Toxocariasis. Brit. Med. J., 3:663-669, 1970.

19. Levine, N. D. Nematode Parasites of Domestic Animals and of Man. Burgess Publ. Co., Minneapolis, Minnesota, 1968.

20. Arther, R. G. Viability and Infectivity of Parasitic Nematode Ova in Anaerobically Digested Sewage Sludge. PhD Thesis, College of Veterinary Medicine, University of Illinois, 1978.

21. Swenson, M. J. Dukes Physiology of Domestic Animals. 4th edition. Cornell University Press, Ithaca, New York, 1977. 914 pp.

22. Peterson, J. Personal correspondence with citation from U.S. Geological Survey analysis, 1978 (1980).

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